National Library of Canada

Acquisitions and Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale du Canada

Direction des acquisitions et des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

Canada

THE UNIVERSITY OF ALBERTA

CORRELATIONS OF PHENOTYPE AND GENOTYPE IN PREVIABLE HUMAN ABORTUSES

by

PUSHPINDER KAUR SEKHON ©

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE
OF

MASTER OF SCIENCE IN EXPERIMENTAL PATHOLOGY

DEPARTMENT OF LABORATORY MEDICINE AND PATHOLOGY

EDMONTON, ALBERTA

SPRING 1995

National Library
of Canada

Acquisitions and
Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale
du Canada

Direction des acquisitions et
des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

THE AUTHOR HAS GRANTED AN IRREVOCABLE NON-EXCLUSIVE LICENCE ALLOWING THE NATIONAL LIBRARY OF CANADA TO REPRODUCE, LOAN, DISTRIBUTE OR SELL COPIES OF HIS/HER THESIS BY ANY MEANS AND IN ANY FORM OR FORMAT, MAKING THIS THESIS AVAILABLE TO INTERESTED PERSONS.

L'AUTEUR A ACCORDE UNE LICENCE IRREVOCABLE ET NON EXCLUSIVE PERMETTANT A LA BIBLIOTHEQUE NATIONALE DU CANADA DE REPRODUIRE, PRETER, DISTRIBUER OU VENDRE DES COPIES DE SA THESE DE QUELQUE MANIERE ET SOUS QUELQUE FORME QUE CE SOIT POUR METTRE DES EXEMPLAIRES DE CETTE THESE A LA DISPOSITION DES PERSONNE INTERESSEES.

THE AUTHOR RETAINS OWNERSHIP OF THE COPYRIGHT IN HIS/HER THESIS. NEITHER THE THESIS NOR SUBSTANTIAL EXTRACTS FROM IT MAY BE PRINTED OR OTHERWISE REPRODUCED WITHOUT HIS/HER PERMISSION.

L'AUTEUR CONSERVE LA PROPRIETE DU DROIT D'AUTEUR QUI PROTEGE SA THESE. NI LA THESE NI DES EXTRAITS SUBSTANTIELS DE CELLE-CI NE DOIVENT ETRE IMPRIMES OU AUTREMENT REPRODUITS SANS SON AUTORISATION.

ISBN 0-612-01651-X

Canada

UNIVERSITY OF ALBERTA

LIBRARY RELEASE FORM

NAME OF AUTHOR: **PUSHPINDER KAUR SEKHON**

TITLE OF THESIS: **CORRELATIONS OF PHENOTYPE AND GENOTYPE IN PREVIABLE HUMAN ABORTUSES**

DEGREE: **MASTER OF SCIENCE**

YEAR THIS DEGREE GRANTED: 1995

Permission is hereby granted to the University of Alberta Library to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes.

The author reserves all other publication and other rights in association with the copyright in the thesis, and except as hereinbefore provided neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the author's prior written permission.

Pushpinder K Sekhon

8507 - 174 Street N.W.
Edmonton, Alberta
CANADA
T5T 2B8

January 9, 1995

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled CORRELATIONS OF PHENOTYPE AND GENOTYPE IN PREVIABLE HUMAN ABORTUSES submitted by PUSHPINDER KAUR SEKHON in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE IN EXPERIMENTAL PATHOLOGY.

Dr. L.H. Honore

Dr. G. Flowerdew

Dr. C.C. Lin

Dr. H. McDermid

Dr. R. Stinson

August 25, 1993

ABSTRACT

This correlative study comparing genotype and phenotype in previable (less than 20 weeks gestational age) human abortuses aimed at expanding the currently recognized phenotypic spectrum associated with documented forms of heteroploidy. The study, which involved 696 archival and prospective cases of diploid and heteroploid previable conceptuses, was based on the accumulation of qualitative descriptive data and the quantitation of conceptal development by anthropometry (measurement of body segments) for the embryo/fetus, and by auxometry (measurement of the growth of the basic unit of the placenta, the single truncal system) and histomorphometry (measurement and analysis of placental structure at the microscopic level) for the placenta. The accumulated qualitative data provided novel descriptions of trisomy 7 and 22 embryos and complete descriptions of non-viable placentae. Furthermore, many specific anomalies were identified which had not been previously documented for their respective heteroploid population over this previable stage in gestation. Using anthropometry, it was possible to assess growth coordination, growth rate changes and variability in a series of parameters by comparison with critical growth indicators. Distinct differences from normal were identified in all experimental populations (trisomy 21, 45,X, and 3n) except for trisomies 13 and 18 when compared to structurally normal fetuses of similar developmental stages. In all cases, anthropometric findings affirmed recognized trends with respect to the physical development of the specific heteroploid populations.

Similarly, placental auxometry was conducted with the aid of single truncal systems (STS) obtained from the chorial plate. Each STS was carefully measured and used to reveal possible changes in the degree of placental thickness and truncus branching. Placental histomorphometry was conducted using a semi-automated, interactive computer program (Genias 25, by Joyce Loebl) to quantitate subtle changes in placental development at the histological level in trisomies 15, 16, and 22, as compared to normal.

As expected, the findings were indicative of a relationship between placental maldevelopment and poor conceptal prognosis. The correlations achieved by all levels of analysis in this study are important in expanding the phenotypic spectrum of documented forms of heteroploidy, thereby allowing more accurate recognition of such heteroploid conceptuses.

ACKNOWLEDGMENTS

I wish to express my heartfelt gratitude to the following individuals, without whom this thesis would not have been possible:

To Dr. Louis H Honoré, my supervisor, who provided me with support and guidance throughout.

To my committee members Drs. Gordon Flowerdew, Heather McDermid, Jim (C.C.) Lin, and Robert A. Stinson who have provided me with insight regarding this project and a careful review of this thesis.

To Dr. Terry Taerum, Carol Smith, Tom Turner and Richard Golda for their much needed technical assistance, and to Dr. David Rayner who shared his insight and provided me with an opportunity to exchange ideas.

To my family for their patience, love, understanding and most of all, for their typing skills.

TABLE OF CONTENTS

CHAPTER		PAGE
1. INTRODUCTION		1
	1.1 PHENOTYPE- GENOTYPE CORRELATIONS	1
	1.2 OBJECTIVES AND TOOLS	1
	1.3 PHENOTYPIC VARIABILITY	2
2. LITERATURE REVIEW		3
	2.1 INTRODUCTION	3
	2.2 QUALITATIVE STUDIES ON EMBRYO/FETAL AND PLACENTAL MORPHOLOGY	5
	2.3 QUANTITATIVE STUDIES I: ANTHROPOMETRY	17
	2.4 QUANTITATIVE STUDIES II: PLACENTAL MORPHOLOGY	22
3. MATERIALS AND METHODS		24
	3.1 INTRODUCTION	24
	3.2 CYTOGENETICS	24
	3.3 QUALITATIVE MORPHOLOGIC ANALYSES	25
	3.4 QUANTITATIVE MORPHOLOGIC ANALYSES	26
4. CYTOGENETICS AND ABORTION		31
	4.1 INTRODUCTION	31
	4.2 EXPERIMENTAL PROTOCOL	31
	4.3 RESULTS	31
	4.4 EVALUATION OF KARYOTYPING EXPERIENCE	32

5. QUALITATIVE FINDINGS AMONGST KARYOTYPED ABORTUS POPULATIONS 34

5.1 INTRODUCTION 34
5.2 EXPERIMENTAL PROTOCOL 34
5.3 RESULTS 34
5.4 INTERPRETATION OF RESULTS 34

6. ANTHROPOMETRY IN THE PREVIABLE STAGES OF HUMAN DEVELOPMENT 70

6.1 INTRODUCTION 70
6.2 EXPERIMENTAL PROTOCOL 70
6.3 RESULTS 73
6.4 INTERPRETATION OF RESULTS 90

7. ALTERED PLACENTAL MORPHOMETRY REVEALED BY TWO DIMENSIONAL IMAGE ANALYSIS 98

7.1 INTRODUCTION 98
7.2 EXPERIMENTAL PROTOCOL 98
7.3 RESULTS 100
7.4 INTERPRETATION OF RESULTS 103

8. GENERAL DISCUSSION 105

8.1 PHENOTYPE-GENOTYPE CORRELATIONS: EXPANDING THE SPECTRUM 105
8.2 THE SCIENCE OF ANTHROPOMETRY AND ITS IMPLICATIONS TO POST-MORTEM ANALYSIS OF THE PREVIABLE CONCEPTUS 106
8.3 QUANTITATION OF PLACENTAL PATTERNS 108
8.4 AMPLIFIED DEVELOPMENTAL INSTABILITY VS. SPECIFIC GENE EFFECT 109
8.5 CONSEQUENCES OF THIS PROJECT 109

9. CONCLUSION 110

BIBLIOGRAPHY 111

APPENDIX A: DATA COLLECTION FORMS 123

APPENDIX B: SECOND AND THIRD DIMENSION ANTHROPOMETRIC PARAMETERS 130

APPENDIX C: CORRELATION COEFFICIENTS OF BMI AND PI 131

APPENDIX D: HISTOMORPHOMETRY PARAMETERS 132

APPENDIX E: FORMAL PROOF OF THE IRREGULARITY FACTORS 133

LIST OF TABLES

Table 2.1: Documented Studies Using Cytogenetics Without Description of Conceptal Phenotype 4

Table 2.2: Cytogenetics Studies Providing Qualitative Descriptions of the Conceptus 7

Table 2.3: Documented Phenotypes of Aneuploid Conceptuses 8

Table 2.4: Documented Phenotypes of Polyploid Conceptuses 13

Table 2.5: Descriptions of Placental Development Based on Genotypic Classification 15

Table 2.6: Anthropometric Data from Abortuses and Term Conceptuses 18

Table 2.7: Anthropometric Data from Heteroploid Abortuses and Term Conceptuses 21

Table 3.1 Anthropometric Databases 29

Table 4.1: Frequencies Of Genotypic Classes Obtained From Human Abortuses 33

Table 5.1: An Overview of Human Abortal Cases Employed in this Study 35

Table 5.2: Compiled Phenotypes of Observed Diploid Cases 36

Table 5.3: Compiled Phenotypes of Aneuploid Cases Examined in This Study 38

Table 5.4: Compiled Phenotypes of Polyploid Cases Examined in This Study 46

Table 6.1: Anthropometric Populations and CRL 71

Table 6.2: Linear Anthropometric Parameters 72

Table 6.3: Pearson Correlation Coefficients For Normal Data (N = 302) 75

Table 6.4: Levene's Test For The Measurement Of Variance Amongst Parameters of a Single Population 89

Table 7.1: Mean Ratios of STS Length, STS Width, and STS Length/STS Width in N and Heteroploid Development 101

Table 7.2: Summary of Histomorphometric Means of Various Parameters Taken Over Control and Study Populations 102

LIST OF FIGURES

Figure 6.1: Normal Anogenital Distance in Fresh Males and Females 84

Figure 6.2: Ear Length in Trisomy 21 vs. Normal 86

Figure 6.3: Upper Arm Length in Trisomy 21 vs. Normal 87

Figure 6.4: Forearm Length in Trisomy 21 vs. Normal 88

Figure 6.5: Ear Length in Monosomy X vs. Normal 91

Figure 6.6: Ear Width in Monosomy X vs. Normal 91

Figure 6.7: Chest Circumference in Monosomy X vs. Normal 92

Figure 6.8: Total Upper Limb Length in Triploidy vs. Normal 93

Figure 7.1: The Mechanics of Semi-Automated Interactive Histomorphometry 99

LIST OF PLATES

PLATE I	54
PLATE II	56
PLATE III	58
PLATE IV	60
PLATE V	62
PLATE VI	64
PLATE VII	66
PLATE VIII	68

LIST OF ABBREVIATIONS

AC: abdominal circumference
AGD: anogenital distance
ASD: atrial septal defect
BMI: body mass index
BPD: biparietal diameter
BW: body weight
CAL: cumulative arm length
CC: chest circumference
CFH: craniofacial height
CH: cranial height
CHL: crown-heel length
CHM: complete hydatidiform mole
CNS: central nervous system
CRL: crown-rump length
DA: developmental age
DAbuvps: infraumbilical length
DBeloxs: supraumbilical length
E: embryo
EarL (EL): ear length
EarW (EW): ear width
EDA: estimated developemental age
F: fetus
EgL: phallus length
EgW: phallus width
F: fingers
FA: forearm
FacArea: facial area
FCS: fragment of chorionic sac
FemurL: femur length
FH: facial height
FL: foot length
FW: facial width
GA: gestational age
GD: growth disorganization
GI: gastrointestinal tract
H: hand
HeadC (HC): head circumference
ICC: intravillous cytotrophoblastic cells
ICD: intercanthal distance
ICS: intact chorionic sac
IND: internipple distance
Int lim: interlimb ratio
IPD: interpupillary distance
IUGR: intrauterine growth retardation
Larm: left arm
LHH: Louis H. Honoré, MB
LL: lower leg
MA: menstrual age
Mouth: mouth width
NasInd: nasal index
NE: nodular embryo
NeckL (NeL): neck length
NeckW (NeW): neck width
NoseL (NL): nose length
NoseW (NW): nose width
NRC: nucleated red cells
NTD: neural tube defects
OA: ovulation age
OD: orbit diameter
OFD: occipitofrontal diameter
PC: pelvic circumference
PHM: partial hydatidiform mole
PI: ponderal index
RCS: ruptured chorionic sac
RH: right hand
SA: spontaneous abortus(es)
SPSS: Statistics Package for Social Sciences
STS: single villous truncal system
T: thigh
TA: therapeutic abortus(es)
TLLL: total lower limb length
TR: trunk length
TULL: total upper limb length
Uarm: upper arm
Ulip (UL): upper lip length
VSD: ventral septal defect

CHAPTER 1

INTRODUCTION

1.1 Phenotype-Genotype Correlation

The relationship between genotype and phenotype presents multiple levels of complexity which are not easily assessed in human development. Although it is known that a strong correlation exists between phenotype and genotype, the mechanism by which the genotype is translated into a three dimensional expression is not understood.

Ultimately, biology aims to understand exactly this. However, in order to do so it is necessary to document meticulously normal and abnormal phenotypes along with their variations. Generally, the recognition of "normal" is easily achieved, and by comparison it is possible to identify the abnormal. Using these observations, it is possible to associate phenotype and genotype, and to ascribe specific expressions to specific forms of heteroploidy.

Two purposes are served by phenotype-genotype correlations. First, the theoretical importance of these correlations is to provide insight into varieties of abnormal phenotypes and the mechanisms which lead to the three-dimensional, functional and structural expression of heteroploidy. Second, their practical importance is in clinical and pathologic diagnosis. The better defined the phenotype of a particular form of heteroploidy, the easier it is to recognize and thus the more accurate is the diagnosis based on examination of the conceptus. This is particularly important now when karyotyping is less available due to budgetary constraints.

1.2 Objectives and Tools

This study focuses on the meticulous documentation of qualitative changes associated with heteroploidy in previable (less than 20 weeks' gestational age) human abortuses, and quantitation of selected aspects of conceptal development, in order to extend currently existing descriptions of these conceptuses. The principal tools which will be employed in the quantitation of conceptal development are anthropometry, auxometry, and histomorphometry.

A comparative anthropometric analysis which utilized measurements of a variety of body segments to assess growth coordination was conducted on the embryo/fetuses. Distinct changes affecting the rates of development of several body segments were identified among different heteroploid populations.

Similarly, comparative auxometric analyses were conducted using the basic unit of the placenta, the truncal system. The single truncal system (STS) is an isolated villus tree which has been dissected from the placenta by clipping it either at its point of attachment to the chorial plate, or with surrounding chorion (Castellucci et al, 1990). In either case, the isolated STS is ensured to be free of branches belonging to other systems, the

concerted effort of which enables the placenta to function in the role of materno-fetal exchange.

Each STS was measured for length and width, the findings were correlated to heteroploid populations and compared to a normal population of similar developmental period. Further morphometric analysis (histomorphometry) of selected placentae occurred at the histologic level where cellular structures were measured to assess for changes related to heteroploidy. The findings demonstrated specific quantitative changes existing amongst control and experimental populations.

None of these techniques reflect novel usage with respect to the material being analyzed. Rather, this analysis either involves an earlier period of development, or a more detailed examination of the respective tissue(s).

One of these tools, placental histomorphometry, is still finding its 'niche' in experimental usage. Although it has been used extensively with a variety of tissue types, its use on placental tissue has been restricted to term placentae. Thus, this study will serve two functions: it will provide quantitative histomorphometric data from four genotypic human previable conceptal populations, and as a result, it will permit critical evaluation of the traditional protocol used in this and similar studies.

1.3 Phenotypic Variability

Within the "normal" population, variation exists which prevents all normally developed conceptuses from looking absolutely alike but which enables them to look recognizably normal. The same variation is seen in heteroploid development, such that no two cases of similar genotype ever look exactly alike. Rather, each case is unique and in order to identify a particular type of heteroploidy there must exist some specific traits that identify that group. However, as the human genome has a limited repertoire of structural expression, individual defects are variably shared by abnormal diploid and heteroploid conceptuses and thus have no diagnostic specificity, singly or in limited clusters. It is mainly this overlap of shared defects that makes accurate clinical recognition difficult and the problem is compounded by phenotypic variability. Thus we must rely on certain clusters of defects to recognize a particular syndrome, whether due to structural/numerical abnormalities detected by classical cytogenetics or due to more subtle abnormalities detected only by molecular techniques. It is obvious that the more detailed the documentation of a particular phenotype, the more accurate will be its recognition and characterization. The main purpose of this thesis is to expand the phenotypic spectrum of selected forms of heteroploidy by using qualitative and quantitative methods in an attempt to improve the accuracy of clinical diagnosis. This may help to shift the emphasis in clinical practice from expensive cytogenetics and molecular genetics.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The review will concentrate on literature documenting qualitative and quantitative early human conceptal development; data on the embryo/fetus will be obtained from the previable spontaneous abortus population in order to identify the early relationship between genotype and phenotype. This area of research is largely incomplete. Many qualitative studies focus on either the embryo or placenta, rarely on both and most are not very detailed. Similarly, quantitative data are available for few parameters on second trimester fetuses and term placentae. Hence, it should not be surprising that most of the research covered in this thesis is not addressed in the literature review.

As a final note, a thorough attempt has been made in compiling this review although the literature dealing with ultrasonography is but a fragment of the entirety. This segment of the literature review is not truly relevant to this thesis since the material (live fetus, *in utero*) is not affected by the same process as the material of this thesis.

In 1961, before the advent of banded mammalian chromosomes were known, the first instance of chromosomal anomaly was documented in a spontaneous abortion (Penrose and Delhanty, 1961). Table 2.1 outlines the larger of the numerous studies which have been conducted showing the strong correlation between spontaneous abortion and heteroploidy. While these studies provide important epidemiological data for numerous forms of heteroploidy, they contribute little information to the pathology of spontaneous abortion and phenotype-genotype correlation since they neglect to include descriptions of the analyzed cases.

The availability of techniques that would permit the absolute identification of chromosomes and hence, genotype, based on gross morphology was the turning point in the field of medical genetics as it relates to cytogenetics and reproductive pathology. This did not come until 1970, when Caspersson *et al.* successfully banded human chromosomes with a fluorescent DNA binding agent, in a technique widely known as Q-banding.

The realization that the presence of an additional chromosome to the balanced genome is capable of generating deviations from normal has led to widespread speculation regarding the origin of the altered phenotype. In 1970, Shapiro presented a model of "amplified developmental instability" outlining the pathogenesis of heteroploid phenotypes specifically formulated on the types and severity of abnormalities that constitute the trisomy 21 phenotype. Shapiro (1983) contends that nature has evolved a co-adapted gene pool in the balanced genome which is disrupted by the presence of a single extra chromosome.

The disruption results, not from the specific excess gene product identified by the loci on that chromosome, but from the metabolic interactions of those gene products with

Table 2.1: Studies Describing the Association Between Spontaneous Abortion and Heteroploidy, Without Specific Descriptions of Conceptal Phenotype

Reference	Sample Size	Successful Cultures	Heteroploid	
			Number	Frequency
Ruzicska & Czeizl, 1970	103 SA 1871 SA*	55 955	20 238	36.0% 24.9%
Lauritsen *et al.*, 1972	100	68	34	50.0%
Boué & Boué, 1973	NA	1457	892	61.0%
Kajii *et al.*, 1973	216 (SA)	152	82	54.0%
Carr, 1977	NA	NA	NA	NA
Hassold *et al.*, 1978	488	234	109	46.6%
Warburton *et al.*, 1979	600 (SA)	592	103	17.0%
Hassold *et al.*, 1980	1120	1000	463	46.3%
Warburton *et al.*, 1980	967	876	287	32.7%
Lin *et al.*, 1985	428 (SA)	215	80	37.2%
Eiben *et al.*, 1987	140 (SA)	140	68	48.6%
Eiben *et al.*, 1990	750 (SA)	750	380	51.0%

* Denotes data compiled from previous studies.

those from other loci throughout the genome (Shapiro, 1983). Furthermore, the gene pool which regulates individual traits, also serves to buffer genetic expression from both genetic and environmental disruptions. The buffering system has been likened to the canalization (Waddington, 1942) of development and also to developmental homeostasis (Lerner, 1970), which are two similar processes describing the ability of the balanced genome to tolerate disruptions caused by genetic and environmental forces. When such forces cannot be overcome, the buffering system is thought to fail, leading to an alteration in phenotype.

Specifically, amplified developmental instability in trisomy 21 (Shapiro, 1983) was thought to result from a generalized disruption of metabolic interactions involving gene products of the entire genome, hence, influencing genetic balance. This contributes to the inability of the system to buffer against the genetic and environmental forces which, in turn, disrupts developmental homeostasis in the conceptus. Shapiro contends that traits expressing a lower degree of canalization demonstrate increased variability, and that the lack of homeostatic control renders them increasingly susceptible to developmental disruptions as caused by the presence of and additional chromosome 21.

Consequently, the model of amplified developmental instability (Shapiro, 1983) predicts that in trisomy 21 (although this would also be true of any other form of trisomy), reduced canalization results in increased variance in metric traits, greater instability in developmental and physiological pathways, and increased susceptibility to genetic and environmental forces. This model has been supported by a number of findings involving human dentition (Shapiro, 1970), palatal dimensions (Shapiro, 1975), dermatoglyphics (Shapiro, 1975) and additional phenomena.

However, there also exists an opposing view to amplified developmental instability, which is founded on reductionist theory suggesting that the individual loci on chromosome 21 contribute to the observed phenotype in a specific and dosage dependent manner (Epstein, 1986; Epstein, 1988). Epstein believes that the combined effect of the increased dosage of all genes represented on the trisomic chromosome contribute to the observed phenotype. The greatest support for this theory is found to be related to the recent mapping of loci on chromosome 21 and the ability to identify phenotypes associated with imbalances of the mapped regions (Tippet and Kaplan, 1985). Regardless, the validity of either Shapiro's or Epstein's theory can only be demonstrated when sufficient data are available.

2.2 Qualitative Studies on Embryo/Fetal and Placental Morphology

The advent of modern, thorough karyotyping opened the door to phenotype-genotype correlation; the conceptal phenotype is derived from the combined analysis of embryo/fetus and placenta. The bulk of literature including textbooks describe phenotype-genotype correlations in the stillborn, liveborn infants and adults (Nora and Fraser, 1989), with little attention paid to previable abortuses.

Genetic abnormalities generally lead to a broad phenotypic spectrum. In early human development, the continuum is represented at one extreme by near-normal development, progressing to include formed embryos and fetuses with focal anomalies

and, at the other end growth disorganized (GD) embryos characterized by partial or total failure of formation of the human body. Variations in growth disorganized embryos have led to a classification system based on the degree of differentiation of the embryo (Poland and Miller, 1973).

GD_1: empty, intact chorionic sac with absence of yolk sac, body stalk, and NRC

GD_2: amniotic sac and solid embryonic tissue without recognizable external features; embryo is attached to the chorion with evidence of a body stalk; disproportionately large chorionic sac

GD_3: grossly disorganized embryo (<10mm CRL) showing recognizable external features such as retinal pigment which allow identification of cranial and caudal poles; absence of limb buds

GD_4: embryos with severe and multiple anomalies (>10mm CRL); identifiable head, trunk and limbs; difficulty in placing in the developmental horizon.

The development of growth disorganized embryos is typically observed with the non-viable forms of heteroploidy. Consequently, it is apparent that the phenotypic spectrum of these heteroploid populations is far more restricted than that of the potentially viable forms of heteroploidy.

Consequently, in certain potentially viable aneuploid populations like trisomies 13, 18, and 21, there is more documentation and the phenotypic spectrum is wider. The phenotypes of later trisomies 13, 18, and 21 abortuses tend to parallel those of their liveborn counterparts (Stephens and Shepard, 1980). Some of the larger studies assessing this correlation are outlined in Table 2.2. Documented phenotypes of trisomies 13, 18, 21, and other forms of heteroploidy are presented in Tables 2.3 and 2.4.

Generally, it has been demonstrated that autosomal trisomies affect embryonic development to a variable degree. The phenotypes of trisomies 3, 5, 12, and 17 are not described in the literature, probably because these trisomies are rarely recovered owing to abortion in very early pregnancy with the exception of trisomy 1 which has never been recovered (Boué *et al.*, 1976; Creasy *et al.*, 1976; Ohama, 1977; Warburton *et al.*, 1980). Rare trisomies, like trisomies 2, 6, 11, 19 and 20 show mostly blighted ova with developmental arrest at approximately 3 weeks developmental age (Boué *et al.*, 1985).

The most common trisomy in spontaneous abortion (Creasy *et al.*, 1976; Hassold and Jacobs, 1984), trisomy 16, also shows primitive development with early developmental arrest giving a characteristically small chorionic sac containing a blob of embryonic tissue arrested at the disc stage (Boué *et al.*, 1985). More advanced, although still abnormal embryonic development is seen with trisomies 4, 5, 7-10, and 22. These trisomies give rise to severely growth disorganized embryos (25-35 days developmental age) which have been noted to have undergone necrosis of certain embryonic cell lines

Table 2.2: Cytogenetics Studies Providing Qualitative Descriptions of the Conceptus

Study	Sample Size	Successful Cultures	Heteroploid	Qualitative Description (E/F, Placenta)
Carr, 1969	9	9	8	Placenta
Philippe & Boué, 1969	206	206	112	Placenta
Mikamo, 1970	150	67	17	E/F
Carr, 1971	29	29	11	E/F
Kuliev, 1971	88	88	18	E/F
Creasy and Crolla, 1974	724	724	24	E/F
Boué *et al.*, 1975	1500	1498	921	E, Placenta
Boué *et al.*, 1976		86	66	E/F, Placenta
Creasy *et al.*, 1976	2620	941	287	E/F, Placenta
Honoré *et al.*, 1976	112	112	112	Placenta
Geisler and Kleinebrecht, 1978	166	166	65	E/F, Placenta
Kajii *et al.*, 1978	944	910	23	E/F, Placenta
Byrne and Warburton, 1979	15	8	6	E/F, Placenta
Kajii, 1980	639	447	241	E/F
Poland *et al.*, 1981	287	188	109	E/F, Placenta
Byrne and Blanc, 1985	879	13	13	E/F
Byrne *et al.*, 1985	3472	1356	540	E/F
Tenti and Rehder, 1985	357	357	357	E/F
Gilbert *et al.*, 1987	NA	NA	NA	E/F, Placenta
Kalousek, 1987	1794	582	460	E/F

NA = Data not available.

Table 2.3: Documented Phenotypes of Aneuploid Conceptuses

Genotype	Description	Reference
Trisomy 1	Never observed. No documented phenotype.	Boué *et al.*, 1985
Trisomy 2	developmental arrest at 3 weeks; typically no embryonic formation - empty sacs; one case with yolk sac, embryo measuring 1mm, DA between 3 and 4 weeks, with NRC; hydatidiform mole	Kuliev, 1971; Kleinebrecht and Geisler, 1975; Creasy *et al.*, 1976; Boué *et al.*, 1985
Trisomy 3	rarely observed; intact empty sac	Creasy *et al.*, 1976; Boué *et al.*, 1985
Trisomy 4	developmental arrest between 25-35 days; disorganized embryos; ruptured sacs	Creasy *et al.*, 1976; Boué *et al.*, 1985
Trisomy 5	rarely observed; developmental arrest between 25-35 days; disorganized embryos	Boué *et al.*, 1985
Trisomy 6	rarely observed; no documented phenotype	Boué *et al.*, 1985
Trisomy 7	developmental arrest between 25-35 days; disorganized embryos; fetus with single umbilical artery, cleft palate, hypoplastic mandible, right descending aorta, malrotation of the gut; small kidney and adrenal, micropenis	Boué *et al.*, 1985; Byrne and Blanc, 1985
Trisomy 8	developmental arrest between 25-35 days; ruptured empty sac; intact empty sac with cord stump; disorganized embryos	Creasy *et al.*, 1976; Boué *et al.*, 1985
Trisomy 9	developmental arrest between 25-35 days; ruptured empty sac with cord stump; disorganized embryos	Creasy *et al.*, 1976; Boué *et al.*, 1985
Trisomy 10	developmental arrest between 25-35 days; ruptured empty sac; disorganized embryos	Creasy *et al.*, 1976; Boué *et al.*, 1985
Trisomy 11	developmental arrest at 3 weeks; rarely observed; blighted ova without embryonic formation	Boué *et al.*, 1985
Trisomy 12	rarely observed; no documented phenotype	Boué *et al.*, 1985

Trisomy 13	Embryos: - developmental arrest between 40-45 days; ruptured sacs with or without cord stump; GD_4 embryos; underdevelopment of the nasal process leading to cleft lip and palate and cyclopia Fetuses: - microcephaly; holoprosencephaly, arhinencephaly, proboscis facial clefting, hypotelorism; microphthalmia; absent uvula; omphalocele, micropenis; cystic hygroma - short limbs, polydactyly - internal anomalies - absence of olfactory bulbs, cardiac irregularities: dextrocardia, mitral valce atresia, bicuspid aortic valve; abnormal lobation of lungs; hypoplasia of lungs; pleural effusion; horseshoe kidney, hydronephrosis; Meckel's diverticulum; malrotation of bowels; muscular anomalies	Poland and Miller, 1973; Calacino and Peltersen, 1978; Kalousek and Poland, 1984; Boué *et al.*, 1985; Byrne and Blanc, 1985; Petrikovsky *et al.*, 1991
Trisomy 14	developmental arrest between 40-45 days; embryonic development ranges from ruptured sacs with cord stump to GD_3 embryos to normal embryos; focal anomalies: underdevelopment of the nasal process leading to cyclopia	Poland and Miller, 1973; Creasy *et al.*, 1976; Boué *et al.*, 1985
Trisomy 15	ruptured empty sac; ruptured sac with cord stump; incomplete embryo; normal fetus	Creasy *et al.*, 1976; Boué *et al.*, 1985; Byrne *et al.*, 1985
Trisomy 16	blighted ova characterized by a chorionic vesicle (2-3 mm), small amnion, tiny embryonic formation (<1 mm) arrested at disc stage; more advanced embryonic development includes GD_1, 4mm GD_2 and 4 documented cases of normal embryos	Kuliev, 1971; Poland and Miller, 1973; Creasy *et al.*, 1976; Boué *et al.*, 1985
Trisomy 17	rarely observed; GD_1 and GD_2 embryos	Boué *et al.*, 1985; Byrne *et al.*, 1985

Trisomy 18	Embryos: - ruptured sacs with or without cord stump; severely disorganized to normal embryos Fetuses: - edema; hydrocephalus, prominent occiput, oxycephaly, hypertelorism, microphthalmia, epicanthal folds, lowset ears, periauricular skin tag; absent external ear; cleft lip and palate, microtia, high palate, micrognathia; cystic hygroma; short fleshy limbs, clinodactyly, polydactyly, syndactyly, simian creases, talipes equinovarus, rockerbottom feet; club feet; short sternum, omphalocele, prune belly, diastasis recti, genital hypoplasia; micropenis; single umbilical artery; normal fetus - internal anomalies - tracheo-esophageal fistula; cardiomegaly, microcardia, ASD, VSD, mitral atresia, stenosis of the ductus arteriosis, tubular hypoplasia of the descending aortic arch, bicuspid pulmonary valve, omphalocele, Meckel's diverticulum, Reidel's lobe of liver, absence of left umbilical artery, renal agenesis, bification of xiphisternum, bicornuate uterus;cryptorchidism	Lewis, 1964; Creasy *et al.*, 1976; Byrne and Warburton, 1979; Boué *et al.*, 1985; Byrne and Blanc, 1985; Byrne *et al.*, 1985; Ville *et al.*, 1992
Trisomy 19	rarely observed; no documented phenotype	Boué *et al.*, 1985
Trisomy 20	range from ruptured empty sac to incomplete embryo to normal fetus	Creasy *et al.*, 1976; Boué *et al.*, 1985; Byrne *et al.*, 1985

Trisomy 21	Embryos: - developmental arrest between 6 and 7 weeks; embryonic range from ruptured sacs with or without stump, to disorganized embryos (2,3), to macerated formed embryos Fetuses: - flat profile, hypotonia, hyperflexibility; holoprosencephaly, oblique palpebral fissures, ocular anomalies, hypertelorism, flattened nasal bridge, micrognathia, protuberant tongue, dysplastic ear, folded upper aural helix; excess nuchal skin; cystic hygroma; short limbs, sirenomelia, limb contractures, webbing, dysplastic middle phalanx of middle finger, transverse palmar crease, triangular middle phalanx on fifth finger, clinodactyly, simian crease, sandal gap; protruding abdomen -internal anomalies - ASD, VSD, single atrium; Epstein anomaly, dysplasia of the tricuspid valve, endocardial cushion defects, pleural effusion; malrotation of the intestines	Lee and Jackson, 1972; Creasy and Crolla, 1974; Creasy *et al.*, 1976; Ginsberg *et al.*, 1977; Byrne and Warburton, 1979; Pi *et al.*, 1980; Stephens and Shepard , 1980; Boué *et al.*, 1985; Byrne *et al.*, 1985; Petrikovsky *et al.*, 1991
Trisomy 22	Embryos: - developmental arrest between 25-35 days; disorganized embryos Fetuses:(2) - IUGR; holoprosencephaly, microcephaly, prominent occiput, epicanthal folds, broad nasal bridge, low set malformed ears, long philtrum, micrognathia, cleft lip and/or palate, finger-like thumb, clinodactyly, syndactyly, genital hypoplasia -internal anomalies: VSD, malrotation of colon, anisosplenia, renal agenesis, single umbilical artery	Boué *et al.*, 1985; Byrne *et al.*, 1985; Kukolich *et al.*, 1989; Isada *et al.*, 1990
Monosomy 21	ruptured empty sac	Kajii *et al.*, 1980

Monosomy X	Embryos: - range from intact or ruptured empty sacs to ruptured chorionic and amniotic sac with fragment of macerated embryonic tissue at distal end of umbilical cord, to disorganized embryos (1-3) to formed and normal embryos - formed embryos may show NTD (encephalocele), cleft palate and/or cleft lip; growth consistent with 5-6 week developmental delay - hydatidiform mole Fetuses: - lymphedema; amniotic bands, anencephaly, spina bifida, hydrocephalus, cyclopia, cystic hygroma, short limbs, polydactyly, syndactyly, amelia, sirenomelia, caudal regression, omphalocele, genital hypoplasia, single umbilical artery - internal anomalies - preductal aortic coarctation, VSD, hypoplastic ventricle, bicuspid aortic valve, persistent left superior vena cava, persistent left cardinal vein, anomalous subclavian artery, mitral valve stenosis, tricuspid valve hyperplasia, ascites, hyperplastic lungs, incomplete lung lobation, single umbilical artery, malrotation of gut, Meckel's diverticulum, short, small bowel, horseshoe kidney, ectopic kidney, asymmetric gonads; -no external or internal anomalies (one case of TA for karyotype)	Mikamo, 1970; Carr, 1971; Foussereau and Philippe, 1972; Poland and Miller, 1973; Boué *et al.*, 1976; Creasy *et al.*, 1976; Geisler and Kleinebrecht, 1978; Fantel *et al.*, 1980; Boué *et al.*, 1985; Byrne and Blanc, 1985; Canki *et al.*, 1988; Kelly *et al.*, 1992; Ville *et al.*, 1992

Table 2.4: Documented Phenotypes of Polyploid Conceptuses

Genotype	Description	Number of cases	Reference
3n Molar	intact empty sacs; ruptured sacs with or without embryos; discrepant growth of amniotic sac and embryo; sac usually contains membranes, cord and embryonic formation; developmental arrest between 4-6 weeks; embryos with severe CNS malformations including: anencephaly, cyclocephaly, absence or anomaly of hypophysis, failure of closure of neural groove, spina bifida; poor limb development; syndactyly	8	Carr, 1971
3n Non-Molar	intact and ruptured empty sacs with or without cord stumps; intact sac containing 0.5 mm NE to GD_4 embryo; embryonic anomalies: encephalocele, NTD, hydrocephaly, frontal dysraphia, talipes, ectopia cordis, pulmonary hypoplasia, renal agenesis; Breus mole	22	Carr, 1969; Kuliev, 1971; Giroud *et al.*, 1973; Boué *et al.*, 1976; Creasy *et al.*, 1976; Boué *et al.*, 1985; Tenti and Rehder, 1985
4n Molar	blighted ova; intact sac no embryo; RCS	4	Carr, 1969; Mikamo, 1970; Boué *et al.*, 1976; Boué *et al.*, 1985
4n Non-Molar	ruptured and intact empty sacs; blighted ova with developmental arrest between 2-3 weeks; conceptuses tend to contain chorionic vesicle without amniotic membrane formation; severely disorganized embryos; severely autolyzed fetus	3	Mikamo, 1970; Kuliev, 1971; Carr, 1971; Boué *et al.*, 1976; Creasy *et al.*, 1976; Boué *et al.*, 1985; Byrne *et al.*, 1985

(Boué *et al.*, 1976; Boué *et al.*, 1985). The result is that other lines continue undifferentiated development, promoting growth disorganization.

Early trisomy 13 abortuses show characteristic hypoplasia of the craniofacies specifically affecting the nasal process; developmental arrest seems to occur at 40-45 days developmental age. Trisomies 14 and 15 generally lead to formation of growth disorganized embryos (Boué and Boué, 1985; Honoré, 1989). In contrast, first trimester trisomy 21 abortuses arrest at 6-7 weeks developmental age and show apparently normal development.

In early pregnancy, autosomal trisomies also affect placental phenotype (Jacobs 1979; Baldwin *et al.*, 1982; Minguillon *et al.*, 1989). The characteristic presentation is one of small, immature villi and marked trophoblastic and vascular hypoplasia (Boué *et al.*, 1976b, Honoré *et al.*, 1976). Although many authors have supplied general descriptions of different types of heteroploid placentae, very few have provided detailed descriptions of such placentae based on more than single cases. Furthermore, the descriptions usually combined different forms of trisomy to give general descriptions of "trisomy" without correlation with specific trisomies. Table 2.5 provides placental descriptions of different genotypic populations.

The greatest alteration in placental phenotype is observed with molar pregnancies, of which there are two types. The first is the partial hydatidiform mole which presents with a combination of normal and hydropic and cystic villi, and is usually associated with the development of fetus, umbilical cord and amniotic membranes. Mostly, partial moles are triploid, although they may result from tetraploid (Surti *et al.*, 1986), trisomic (Vassilakos *et al.*, 1977) and diploid conceptions (Szulman *et al.*, 1978a; 1978b).

In comparison, the complete hydatidiform mole shows marked degenerative changes expressed as large grape-like clusters of villi throughout the placenta with distinct trophoblastic hyperplasia and a tendency toward neoplastic change (Tsuji *et al.*, 1987; Bracken, 1987). It is not compatible with fetal development as evidenced by the complete absence of fetus, cord and amniotic membranes; cases of complete hydatidiform mole accompanied by a fetus have been documented which consistently demonstrate twin, dizygotic gestations (Lawler and Fisher, 1987).

The genetic aetiology of the complete mole stems from dispermic fertilization of an anucleate ovum (Ohama *et al.*, 1981; Saji *et al.*, 1989) or by endoreduplication of the haploid pronucleus of a single sperm on fertilization of an empty ovum or one in which the female pronucleus has been inactivated (Ohama, 1986). Thus, the genome of the complete mole is exclusively paternally derived (Kajii and Ohama, 1977). It appears that a small proportion, between 5-10%, of these conceptions actually arise by dispermy (Ohama *et al.*, 1981; Yamashita *et al.*, 1981; Fisher and Lawler, 1984). As this group of complete moles are prone to neoplastic change in the form of choriocarcinoma (Kajii, 1980) knowledge of the karyotype and genetic origin of the conceptus is critical to its clinical management, and is readily correlated to its phenotype.

Table 2.5: Descriptions of Placental Development Based on Genotypic Classification

Genotype	Placental Description	Reference
Diploid (Normal) early placental development	villi of uniform diameter with symmetrical branching appearance and multiple buds	Kalousek and Poland, 1984
"trisomy"	Generalized hypoplasia, defective truncal development leading to small villi, trophoblastic hypoplasia, vascular hypoplasia in chorial plate and villous axis, intravillous cytotrophoblastic cells; hydropic stromal degeneration adjacent to villi of multiform appearance; flat villous epithelium; few trophoblastic invaginations 3 classes: (1) severe hydropic change throughout the placenta, small stromal trophoblastic buds (2) some clubbed or cystic villi throughout a generally normal placenta (3) apparently normal, diploid appearing placenta; focal hypoplasia of trophoblastic epithelium: severe hypovascularity and hypocellularity, occasional intravillous cytotrophoblastic cell	Boué *et al.*, 1976; Honoré *et al.*, 1976; Boué *et al.*, 1985; Minguillon *et al.*, 1989; Kuhlmann *et al.*, 1990
45,XO	very similar to normal placenta; occasionally Breus mole; small avascular or hypovascular villi; hypoplastic trophoblast; developmental arrest of the placenta seems to precede that of the embryo by 1-2 weeks	Foussereau and Philippe, 1972; Boué *et al.*, 1976; Honoré *et al.*, 1976; Boué *et al.*, 1985

3n Non-molar Molar	Non-molar development: normal or hypoplastic trophoblast without macroscopic villous enlargement or cystic enlargement; occasional Breus mole; avascular, macrocystic villi, cystic change confined to chorion frondosum; deep epithelial invaginations; irregular serrated villous surface with syncytial hyperplasia in trophoblastic invagination; hydropic and vesicular villous change Molar development: large swollen chorial villi, cystic change, some trophoblastic hyperplasia.	Carr, 1969; Honoré *et al.*, 1976; Szulman *et al.*, 1981; Minguillon *et al.*, 1989
4n	severe decidual and intraplacental hemorrage, cystic and clubbed villi, focal acute placental infarction; vascular edematous villi, hyperchromatic nuclei; irregular epithelial width with smooth villous surface; focal large hydropic villi with irregular branching patterns; absence of stem villi differentiation	Honoré *et al.*, 1976; Minguillon *et al.*, 1989
"heteroploidy" including 45XO, trisomy, 3n and 4n	hydropic villus change, fibrosis, atypical stromal cells, lymphocytic aggregates, villus scalloping with trophoblastic invagination	Novak *et al.*, 1988

2.3 Quantitative Studies I: Anthropometry

While focal morphologic changes in development are generally obvious, it is far more difficult to identify subtle changes resulting from disturbances in growth coordination. Anthropometry is a tool which has enabled researchers to analyze changes in growth coordination by a comparison of segmental measurements amongst different populations. In 1986, Meaney and Farrer published a compilation of previously published data which used anthropometry in the field of clinical genetics. Even though the data were derived from only the viable population, this paper was important in recognizing the value of clinical anthropometry to medical genetics, and as an extension to the pathology of the previable abortus.

The original studies conducted on embryos and fetuses date back as far as 1909 when Jackson analyzed prenatal human development focusing on specific body segments and organ volumes. The study was based on 43 embryos and fetuses which he used to measure total body volume; the volumes of the head, trunk, extremities and principle organs (brain, eyeballs, thyroid, thymus, heart, lungs, liver, pancreas, spleen, stomach, kidneys, adrenals, ovaries/testes). The comparison of these specimens was completed on a gender-based level. Jackson found a strong correlation with growth and age for all parameters; males were generally heavier than females by total weight; females tended to have heavier organs than males.

Another study by Streeter (1920) compiled 5 years worth of data on normal embryonic development and used it to construct a growth curve for the human embryo. The parameters measured were weight, CRL, head size (measured as the mean of the head circumference at its greatest point and the biauricular transverse arc), foot length, and menstrual age. Streeter concluded that for the purpose of determining age, weight and CRL show a much stronger relationship to age than does head size or foot length. This confirmed a previous finding (Mall, 1918) based on 1000 cases, that a strong relationship exists between age and CRL. This relationship was later shown to be linear (Scammon and Calkins, 1929).

Since the late 1960's, anthropometric studies have been employed in assessment of growth coordination in aborted embryos and fetuses (Table 2.6 and 2.7). Although general assessments of normal embryonic development exist as in the classical embryology of O'Rahilly (1967), no measurements or anthropometric data were presented. Normative measurements were provided by several large studies, including the landmark study by Nishimura *et al.* (1968) who analyzed 1213 intact embryos, obtained by therapeutic abortion, for four parameters: age, crown rump length, body weight, and developmental horizon. They found a strong correlation amongst these parameters. Interest also shifted to organ weights (brain, liver, lung, kidney, heart, adrenal, thymus, spleen, thyroid) in spontaneous and early therapeutic abortuses (Tanimura, 1971). In comparison to

Table 2.6: Anthropometric Data from Abortuses and Term Conceptuses

Study	Components		*N*	Parameters	Data Equations / Graphs
Iffy *et al.*, 1967	E	4-30 mm CRL	149	CRL, GA	+/+
Jackson, 1909	E/F	6mm to term	43	total volume, head vol., trunk and extrem. vol., each of principal organs (vol.)	+/-
Mall, 1910	E/F	NA	1000	CR, RH, MA, copulation age	+/+
Streeter, 1920	E/F	Early first to late third trimester.	704	CRL, weight, CHL, MA, BPD*, BW *mean of biauricular diameter and greatest horizontal diameter	+/-
Clatworthy & Anderson, 1944	E/F	*derived from data provided by Streeter (1920), ,Jackson (1909) and Scammon (1919, 1926, 1927)		Thymus, Spinal cord, Thyroid, Heart, Head, lungs, alimentary canal, stomach, liver, pancreas, spleen, kidney, adrenals	+/-
Nishimura *et al.*, 1968	E/F	14-55d GA	675	OA, dev. horizon, CRL, BW	-/+
Staflova, 1971	E/F	NA	138	Orbit Diameter	+/+
Tanimura *et al.*,1971	E/F	Range of all trimesters	74	CRL, orbit diameter, head diameter	-/+
Tanimura*et al.*, 1971	E/F	Previable (<500g)	136	Body Wt, organ wts (brain, liver, lung, kidney, heart, adrenal, thymus, spleen, thyroid)	+/-
Iffy *et al.*, 1975	E/F	6-20 weeks GA	783	CRL, FL, BWt, CHL, BPD	+/+
Iffy *et al.*, 1978	E/F	6-20 wks GA	557-602	MA, CRL, BW, BPD	+/+
Moore *et al.*, 1981	E/F	Range of previable and viable.	494	coital age, postural age, CRL	-/+

Scammon & Calkins, 1929	F	4mos to term	>300	71 parameters (non-organ measurements)	-/+
Greenwald & Minh, 1960	F	23-42 weeks GA	3000	BW, CHL, GA, Organ weights (heart, lungs, spleen, liver, adrenal glands, kidneys, thymus, brain)	+/-
Berger *et al.*, 1975	F	15-24 wks GA	476	GA, CRL, BPD	+/+
Berkbeck *et al.*, 1975a	F	8-21 wks	122	CHL, CRL, BPD, FL, BW	+/+
Berkbeck *et al.*, 1975b	F	8-21 wks	149	CRL, OFD, BPD, FL, bideltoid diam, BW	+/+
Golbus & Berry, 1976	F	90-170d post menses	133	linear meas, organ weights, BW	+/+
Jakobovits *et al.*, 1976	F	10-20 weeks GA	822	MA, CRL, FL, BW	-/+
Mukerje, 1980	F	13-36 weeks GA	90	uterus, ovary, GA	+/+
Schulz *et al.*, 1980	F	24-term	638	organs extensive	+/-
Hern, 1984	F	10-26 wks GA	1800	BW, LL, BPD, plac wt, Af vol vs FL	-/+
McBride *et al.*, 1984	F	11-19 weeks GA	100	CRL, HC, CC, IND, ULip, EarL, EarW, UArm, LArm, Hand, ULeg, RLeg, foot, CAL, arm span; leg/CRL, arm/CRL, IND/CC, arm/leg, arm/CC, arm span/CAL, inner canthus/outer canthus, BW, brain, liver, lung, kidney, heart, spleen, thymus, adrenal	+/+
Seeds*et al.*, 1984	F	20-30 wks GA	NA	CRL, BW, Fermur L	-/+
Hadlock *et al.*, 1985	F	Term	109	BW vs Head C, fermurl, AC, BPD	-/-

Deter *et al.*, 1986	F	Viable fetuses (<37 weeks GA)	18	BPD, HC, AC, HSA, ASA, HVol, ASA	-/+
Fitzsimmons *et al.*, 1988	F	12-25 weeks GA	58	large intestine, small intestine, GA, stomach, total GI length, appendix	+/+
Sherwood, 1992	F	15-43 wks	NA	Extensive	+/-
Brenner *et al.*, 1976	F I	8-20 weeks GA 8-20 weeks GA 21-44 weeks GA	496 430 30, 772	FW, MA, CRL, CHC	+/+
Siebert, 1986	F/I	24-40 weeks	30	length and width of cribiform plate , perpendicular plate length, outer orbital distance, interpupillary distance, intercanthal distance, nose height breadth, philtrum, mouth width, tongue l/w/thickness, CHL, ethmoid bone	+/-

Table 2.7: Anthropometric Data from Heteroploid Abortuses and Term Conceptuses

Study	Components		*N*	Parameters	Information	
					Equations	Graphs
Kucera & Dolezalova, 1972	F	28-42 weeks GA; Downs and other malformed	3210	BW, CRL	+	+
Perry, 1984	F	Tris 21	NA	cephalic parameters	-	+
Kurjak & Kirkinen, 1974	F	chromosomal aberrations: tris 13-15 (2) tris 18 (2) tris 21 (8)	12		+	+
Chen *et al.*, 1972	I	Term	422	Genotype-BW	+	-
Shami *et al.*, 1991	In-fants	Term	662	Gestational age, BW, Recumbent length, HC, CC	+	-

body weight, certain organs showed general weight increases (liver, heart, brain and thyroid), and others increased at a much faster rate (thymus, adrenal and spleen). The kidney initially increased in weight and then plateaued, whereas the lung first increased and then decreased in relation to body weight. Yet other studies focused on a specific interval in early development, such as that of Golbus and Berry (1976). They assessed selected growth parameters as in fetal length (crown rump length, crown heel length), body weight, foot length, and organ weights between 90 and 170 days post menses. Again, they were able to demonstrate a strong correlation amongst the parameters analyzed.

Later, the most extensive of such studies, McBride *et al.*, (1984) evaluated 100 therapeutically aborted normal fetuses assumed to be normal clinically and morphologically (no karyotype was available), between 11 and 19 weeks gestational age for body weight, crown rump length, head circumference, chest circumference, internipple distance, philtrum length, ear length, ear width, upper arm length, forearm length, hand, calf length, thigh, foot length, crown ankle length, armspan, and organ weights (brain, liver, lung, kidney, heart, spleen, thymus, and adrenals). Correlative analysis demonstrated tight linear coordination of growth relationships, as expected.

From Table 2.7, it is evident that interest in these data existed through the 1970's. During the 1980's, as interest waned in the pathologic examination of abortuses, most anthropometric data were derived from the field of ultrasonography which has emerged as a quick and reliable method for determining the age of a pregnancy (Pedersen, 1982; Quinlan *et al.*, 1982; Seeds and Cefalo, 1982; Hill *et al.*, 1983).

Although significant research has been done on body parameters and organ weights of normal viable fetuses, infants and children (Coppoletta and Wolbach, 1933; Gruenwald and Minh, 1960; Schultz *et al.*, 1962), comparatively little has been completed in the earlier developmental period of both normal and abnormal conceptuses. This literature review demonstrates that for the series of normal studies conducted in the last century, most provide little new anthropometric information since they largely examine a few select parameters that have previously been analyzed. Furthermore, studies involving heteroploid conceptuses are limited both in number of specimens and parameters analyzed. No comprehensive anthropometric study involving all body segments has been attempted for either the normal or heteroploid population.

2.4 Quantitative Studies II: Placental Morphology

Probably the most neglected of human organs, the placenta is a transient organ which can reveal significant information about the conceptus with predictive value for the identification of abnormal karyotype (Honoré *et al.*, 1976). However, a thorough evaluation of early placental changes is rare (Bruyere, 1987), and there is current debate regarding the value of examining placentae from spontaneous abortion (Fox, 1993).

While few attempts have been made to correlate placental phenotype with genotype (Philippe and Boué, 1969; Philippe, 1973; Boué *et al.*, 1976; Honoré *et al.*, 1976; Kajii *et al.*, 1978; Novak *et al.*, 1988; Minguillon, 1989), even fewer attempts have

been made to identify quantitative placental changes in heteroploidy whether at the gross or histologic level.

In a study by Rehder *et al.*, (1989), the diameter of chorionic villi were measured from diploid, and heteroploid non-growth disorganized missed abortuses. While no significant changes were detected amongst the diameters of chromosomally normal villi between 7 and 16 weeks gestation, this study was able to demonstrate that morphological phenotype does correlate with morphometric and cytogenetic data.

The morphometric study conducted by Kuhlmann *et al.*, (1990) was unique in analyzing aneuploid non-growth disorganized placentae for numbers of secondary and tertiary villi, small muscular arteries, and total vessel counts in a 100x field. The placentae were obtained from aneuploid conceptuses between 18 and 23 weeks gestation, and the counts were done manually, five per case by two "blind" researchers. They were able to conclude that placental structure and function will determine prognosis for these conceptuses. They found no change in the numbers of secondary and tertiary villi, but a significant decrease in small artery and total vessel counts in the aneuploid population.

The study by Kuhlmann *et al.* was critical in laying the foundation for quantitative placental histomorphometry. Currently, newer semi-automated technology is available which will take measurements and conduct correlations under the guidance of a user. Such technology has been evaluated in the morphometric assessment of 200 human term placentae (Franco *et al.*, 1987) for three parameters: outer villous parameter, parenchymal area, and mean villous chord length. From this particular study, it is apparent that this computerized, semi-automated, interactive method is invaluable in obtaining histomorphometric data which can reveal information about the conceptus in addition to the findings in the embryo/fetus.

Although the literature regarding quantitative placental analysis is extremely limited and only involves second, third, trimester and term placentae, it provides reasonable methodologies for the application of similar techniques to earlier conceptuses while, more importantly, demonstrating the value of such techniques to placental quantitation; no literature is available on the quantitation of the previable human placenta whether normal or heteroploid.

CHAPTER 3

MATERIALS AND METHODS

3.1 Introduction

Data collection from archival (specimens obtained prior to September, 1991) and prospective (specimens obtained from September, 1991) human abortuses was completed using a tiered approach. The three tiers consisted of cytogenetic, qualitative morphologic, and formal quantitative analyses. These three approaches were employed to ensure a thorough analysis of selected aspects of human heteroploidy as compared with diploidy in spontaneous abortion. This ensured a comprehensive examination of developmental differences which exist amongst normal diploid and a variety of heteroploid populations.

3.2 Cytogenetics

Six-hundred-ninety-seven cases were involved in this study; 342 specimens were karyotyped. Three-hundred-eight archival cases had been previously karyotyped, whereas some selected prospective specimens were cultured using a technique for primary human fibroblast culture in a shared manner by the Division of Cytogenetics (Department of Laboratory Medicine and Pathology, University of Alberta Hospital) and the student. The non-sterile tissue, most often obtained form the placenta and rarely from fetal muscle, was immediately placed in RPMI 1680 media containing static levels of the antibiotics penicillin and streptomycin (Lin *et al.*, 1985). This step was taken to prevent infection of the cultures. Within 72 hours of incubation in RPMI 1680, the tissue was fragmented with a sterile scalpel and forceps in the presence of 0.5ml Amniomax culture media. Excess media was drawn off the plastic petri dish with a sterile 2.5ml micropipette. After 15-20 minutes 2ml of additional culture media was added and the dish was covered and gently placed in a 37°C, water jacketed incubator calibrated to 5% CO_2. The culture remained undisturbed for seven days, and on the eighth day the culture medium was replaced with 2-3ml of fresh Amniomax media. If at any period during the culture process contamination was evident, the appropriate petri dishes were discarded and the case was recorded as being lost to "infection". However, for all other cases the harvest was initiated.

A. Harvesting From Culture

The harvest of cultures was started by adding 50µl colchicine per plate under sterile conditions. The plates were allowed to incubate for 3-4 hours, afterwhich 4ml distilled water was added to the remaining 1ml of media. This was followed by a 20 minute incubation at 37°C. Next, 0.4ml of fresh fixative (3 methanol : 1 acetic acid) was added and each plate each allowed to sit at room temperature for 10 minutes. Then, 2.5ml

of media/fixative was replaced from each plate with an equal quantity of fresh fixative. Ten minutes later, the solution in the plates was replaced with 5ml of fixative; this step was repeated after 20 minutes, for the same duration.

The final step of the harvest was an acid rinse of the colonies. The fixative was poured off the plate, and replaced with just enough acid rinse (35ml glacial acetic acid : 13ml water : 7ml methanol) to cover the base of the petri dish. It was only permitted to cover the colonies for 6 seconds, afterwhich it was poured off and the plate was first dried uncovered on a slide warmer, and then baked, covered, overnight at 56-60°C.

B. Giemsa Banding

Harvested plates do not take up stain if left unstained after the harvest for great lengths of time. Thus, it was necessary to proceed the next day with Giemsa banding (G-banding) of the preparations using the standard technique (Ross and Gormley, 1973) as follows:

1) 10mg trypsin dissolved in 50ml Hanks Balanced Salt Solution (HBSS); 50-55 seconds
2) Phosphate Buffer (pH = 6.8); quick, vigorous rinse
3) Phosphate Buffer (pH = 6.8); quick, vigorous rinse
4) 5ml Giemsa stain mixed with 50ml Phosphate Buffer (pH = 6.8); 5 minutes
5) Phosphate Buffer (pH = 6.8); quick, vigorous rinse
6) Phosphate Buffer (pH = 6.8); quick, vigorous rinse.

Prior to banding, the rims of the petri dishes were removed and the plates were bisected. Following banding, water droplets were removed from the preparations by blotting and pressurized air. Once dry, the preparations were labelled and mounted on glass slides, for scanning under the light microscope.

C. Karyotyping

All slides were manually scanned using a light microscope. Acceptable metaphase spreads, three per case and having less than three overlapping chromosomes, were photographed using high contrast 35mm Kodak Technical Pan ESTAR-AH Base Professional Black and White Negative Film (TP651). The rolls of film were developed and printed. The photographs were utilized such that one complete karyotype was presented per case, and the chromosomes of 1 or 2 other spreads were labelled on the respective photographs so as to indicate the chromosomal constitution of that conceptus.

3.3 Qualitative Morphologic Analyses

This was achieved at three levels of visualization:

1) at the macroscopic level, fresh tissues were examined with the unassisted naked eye

2) the naked eye assisted by a magnifying glass (8x) or a dissecting microscope (magnification range 7-20X)

3) light microscopy, examining routinely prepared and stained histologic sections on glass slides.

The method of gross examination, developed and modified over the years (Honoré and Robertson, 1985; Curry and Honoré, 1990) was the following:

1) the aborted material, received fresh or in sterile isotonic saline, was washed clean in saline and floated in a "sterile" Petri dish

2) samples of membranes, cord, placenta and embryo/fetus (as available) were taken with clean instruments and washed again in saline before transfer to sterile tubes

3) the tubes were then taken within a half an hour of sampling to the Cytogenetics Laboratory.

4) the rest of the material was examined grossly with a magnifying glass or dissecting microscope looking for placental abnormalities, i.e., hypoplasia, villous clubbing or cystic change, hemorrhage; cord lesions; structural anomalies of the embryo/fetus (intact or fragmented)

5) the embryo/fetus, when available, was examined qualititatively and quantitatively using the naked eye and the dissecting microscope as needed. All external anomalies were carefully looked for and recorded; measurements of body fragments were made using the flexible ruler.

6) for histology,samples were taken from membranes, cord, placenta and embryo/fetus, according to availability.

For data analysis, the method of description used was previously standardized by LHH using archival specimens. Current specimens were examined by the student and LHH and described by LHH and the findings were recorded in pathology reports and on data collection forms.

3.4 Quantitative Morphologic Analyses

A. Introduction

In order to quantitate differences between diploid and heteroploid conceptuses it was necessary to analyze both components of the conceptus, embryo/fetus and placenta, where both were present. Cases that included an embryo/fetus were subjected to an anthropometric analysis to establish the extent of growth coordination and growth rates in comparison with normal. Many of these cases were also subjected to gross analyses of single truncal systems of the placenta and/or placental histomorphometric analyses via an interactive, semi-automated image analysis system.

B. Anthropometry

Cases with embryo/fetus, whether whole or fragmented, were subjected to anthropometric analysis. If the case was not received in formalin and external examination was suggestive of chromosomal anomaly, tissue was taken for primary culture and karyotyping[1]. Some specimens that were not karyotyped were included as fresh structurally normal specimens for anthropometry if they satisfied the following criteria:

1) the absence of gross external and internal anomalies.
2) acute expulsion without intrauterine retention
3) singleton pregnancy,
4) the establishment of the cause of pregnancy failure as a factor extrinsic to the pregnancy, e.g. abruption, ascending infection, or combination of both.

These cases were assumed to be diploid, and the derived data were entered into the normative fresh database. Seven karyotyped cases fulfilling the above criteria were also included in this database.

As many heteroploid conceptuses are frequently retained, a corresponding set of structurally normal macerated embryos and fetuses was collected for comparison. This group of cases, reflecting the effects of maceration on embryonic/fetal structures, was established on the following criteria:

1) maceration due to intrauterine retention following the death of the conceptus,
2) karyotypic result of diploidy, where such analysis was available
3) the establishment of the cause of pregnancy failure as a factor extrinsic to the pregnancy, e.g. cord accident, amniotic bands, placental infarction, abnormal placentation, immune rejection.
4) absence of external and internal anomalies.

All cases used to establish the normative databases were structurally normal, and were either known to be diploid or else assumed to be so by virtue of fulfillment of the specified criteria. The vast majority of heteroploid conceptuses, reaching the embryonic or fetal stage at the time of abortion, show some external and/or internal anomalies; normal looking heteroploid embryos/fetuses are occasionally described in the literature but it is unclear as to what extent and with what degree of expertise these specimens were examined. It is also worth stressing that fetuses without external anomalies have a 1% chance of having significant internal anomalies (Nishimura *et al.*, 1968).

Other cases were included in experimental populations if the corresponding karyotype revealed a form of potentially viable aneuploidy as in trisomies 13, 18, and 21; 45,X or 3n. Consequently, the anthropometric data collection has resulted in the formation of eight databases (Table 3.1).

---

[1] Since this study was not designed to determine the incidence of heteroploidy in spontaneous abortion, but to establish genotype-phenotype correlations, only selected cases were karyotyped. To maximize the yield of heteroploid conceptuses for study, grossly abnormal specimens were preferentially selected.

Measurements for all cases were taken manually with a flexible ruler and were documented either in the form of a case report (archival) and subsequently transferred to data collection forms (Appendix A), or immediately recorded on data collection forms (current). Measurements were taken by the student for 11 fetuses and verified (by LHH) for 9 of the 11. Validation of the flexible ruler, single measurement had been previously performed (Honoré, unpublished). To validate the use of a single ruler measurement, the same measurements were taken with a flexible ruler to the nearest millimeter and compared to those obtained using calipers at another time. The non-significant results indicated that measurements taken with a flexible ruler were as accurate. Since the flexible ruler affords greater convenience, it was employed in this study. The validity of single measurements was similarly tested by repeating the same measurements "blindly" on three different days and recording the results separately. Again, there were no significant differences either between any single values or between the means of the four measurements taken.

As specimens are occasionally received in formalin, it was necessary to determine the effect of fixation on measurements. Several selected segments were measured in a group of fresh fetuses, afterwhich the fetuses were immersed in formalin. Three, seven and fourteen days following fixation, the measurements were repeated. The result demonstrated that formalin fixation causes no statistically significant difference in measurements (Honoré, unpublished).

C Single Truncal Systems

The placenta is easily assessed qualitatively and semi-quantitatively. However, for precise quantitation it is best to reduce the placenta to its basic units - the individual truncal systems. This was achieved by a comparison of the normal length and width of such systems as compared to ones derived from heteroploid conceptuses in 22 cases. The system was obtained with the aid of a dissecting microscope and fine instruments to cut the truncal unit where it is attached to the chorial plate. The image of the system was subsequently captured at low power on 35mm Kodak Ektachrome 160 ET colour slide film. Measurements of acceptable preparations were taken by projecting the slide through a slide projector and onto a screen. By extrapolation the actual truncal system measurements were obtained.

D Image Analysis

The computer program Genias 25 (by Joyce Loebl) was used for systematic placental histomorphometry. It facilitates such analyses as it is an interactive, semi-automated program that permits the user to collect vast amounts of data with little difficulty. The material analyzed consisted of placental samples obtained from the non-viable study populations of 35 cases of spontaneously aborted trisomies 15, 16, and 22, and the control population of partially karyotyped (7/16) therapeutic abortuses; these samples were routinely embedded in paraffin and stained with Hematoxylin and Eosin (H&E).

Table 3.1 Anthropometric Databases

DATABASE	TISSUE	TYPE/ KARYOTYPE	NUMBER
Normative Fresh	F	SA (7)	302
Normative Macerated	M	SA (4)	47
Normative Fragments	F	TA (27)	27
Trisomy 21	F/M	SA (15) TA (26)	41
Trisomy 18	F/M	SA (0) TA(9)	9
Trisomy 13	F/M	SA(3) TA(4)	7
Monosomy X	F/M	SA (12) TA (9)	21
Triploid	F/M	SA (11) TA (4)	15

F = fresh

M = macerated

It used a few simple interactive steps of thresholding, dilating and editing to obtain the image to be analyzed. This was followed by determination of parameters to be measured, and finally the actual measurements. Ultimately the process of capture-edit-measure results in the creation of data files for each image analyzed, containing the specified measurements in units to which the system was calibrated. As with all aspects of the program, Genias 25 also calibrates itself in a semi-automated, interactive fashion. The user simply indicates two points, the distance between which reflects a particular unit-measure, and the computer proceeds with the calibration; the degree of accuracy is improved by having the computer calculate multiple distances.

For each of the specimens available, optimally, ten fields were analyzed by this method, for a total unit area examined per case of $8.6 \times 10\mu^2$. Three cases, however, did not provide sufficient placental samples to permit the analysis of ten fields, hence, a smaller area was analyzed. In each field, care was taken to ensure that no residual hemorrhage, chorion, amnion, free floating sprouts or other debris were included in the measurement process.

CHAPTER 4

CYTOGENETICS AND ABORTION

4.1 Introduction

All specimens selected for cytogenetics were karyotyped by an indirect method, involving short term culture of embryonic, if available, or placental tissue. A detailed protocol is found in section 3.2 and criteria are found in section 4.2.

4.2 Experimental Protocol

As indicated in Chapter 3, any grossly abnormal specimens were subjected to cytogenetic analysis. The purpose of cytogenetic analyses was two fold. First, it was meant to provide necessary genotypic information of the respective conceptuses and, secondly, it was to provide practical experience in harvesting and karyotyping. Over a 17-month period, (September 1991 to February 1993), 46 cases were subject to culture for the purpose of karyotyping. An additional group of ten cases were karyotyped from specimens received prior to September 1991.

4.3 Results

A. Success Rate of Cultures

Success rates of the cultures are assessed in 46 cases since ten of the cases were only karyotyped by the student, but not cultured by the same. Of 46 cases harvested by the student, 12 were discarded due to infection, 10 yielded no results on harvest due to overexposure to hypotonic solution, and 24 were successfully harvested and karyotyped. Thus the overall success rate was 24/46, 52%. This is in comparison to the rate for the lab which is 91.5%.

It was assumed that the source of infection of failed cases was the tissue itself since other comparable cases in the same chamber remained uninfected. However, considering that 39/46 cultures were not set by the student and that nine were discarded due to infection, the adjusted success rate for cultures not set by the student is 30/39, 77%.

The balance of 7 cultures were set by the student. Three of these were discarded due to severe infection, following which the student's protocol was altered to include early incubation of the tissue to be cultured in RPMI 1640 medium containing static levels of the antibiotics penicillin and streptomycin. The inclusion of this step resulted in a success rate of 4/4, 100%.

B. Karyotypes

About half of the karyotypes obtained from these cultures were normal diploid (18/34), while 16/34 were heteroploid. The results revealed mostly numerical errors with

only one structural rearrangement, that of a 45, XO, t(1q:19q). The frequencies of each of the genotypes obtained are included in Table 4.1.

4.4 Evaluation of Karyotyping Experience

The purpose of this component was two fold. First, it was meant to provide karyotypes for genotype-phenotype correlation. Secondly, it was to provide the student with practical experience in tissue culturing and karyotyping allowing the student to gain insight into some of the problems associated with this procedure.

Although the traditional protocol used in culturing the available tissue, whether embryonic or placental in origin, was satisfactory in providing sufficient metaphase nuclei in most cases, two basic problems were encountered. Firstly, infections in many of the cultures necessitated discarding the culture in order to avoid contamination of other specimens sharing the incubator. The source of the infections was likely the tissue itself although the process of tissue infection may have been facilitated by poor handling of the specimen (i.e., not refrigerated) prior to tissue sampling for the purpose of karyotyping. This might have been improved by increasing the dosage of antibiotics in the culture media, but higher levels of antibiotics were not used because they would have interfered with viability and culture.

Second, ten other culture failures were related to overexposure to a hypotonic medium. This could have been avoided with the realization that seasonal influences environmental humidity levels which, when elevated would reduce the time of exposure to hypotonic medium required by the harvest protocol. Such an adjustment would likely have rescued all cultures which succumbed to this technical error.

Table 4.1: Frequencies Of Genotypic Classes Obtained From Human Abortuses

KARYOTYPE	NUMBER	FREQUENCY
46, XX	14	0.41
46, XY	4	0.12
45, XO	5	0.15
47, XX, +6	1	0.03
47, XX, +10	1	0.03
47, XX, +14	1	0.03
47, XX, +15	1	0.03
47, XX, +16	2	0.06
47, XX, +22	1	0.03
3n	2	0.06
4n	2	0.06

CHAPTER 5

QUALITATIVE FINDINGS AMONGST VARIOUS ABORTUS POPULATIONS

5.1 Introduction

The purpose of the cytogenetic analyses was two fold, i.e., for clinical diagnosis and for the correlation of phenotype with genotype. To do this in the previable abortus, it is necessary to compile the range of phenotypes for each genotypic population in an effort to describe the observed range of associated phenotypes and to expand the known phenotypic spectrum.

5.2 Experimental Protocol

The data collection forms (Appendix A) were used to extract qualitative data from the pathology reports of archival (before September, 1991) and prospective (since September, 1991) specimens. The total number of karyotyped cases was 342 as indicated in Table 5.1.

5.3 Results

The data, compiled by genotype, are presented in Table 5.2 for the diploid population, and Table 5.3 for the aneuploid, and Table 5.4 for the polyploid population. As expected, phenotypic variations were observed for both embryonic and placental development, except when only single cases were available for analysis as in cases of trisomies 10, 17 and 19. Due to the small numbers of cases, and the quality of specimens, attempts could not justify the determination of frequencies of the specific phenotypes.

5.4 Interpretation of Results

A Diploid

The ranges of phenotypes were fairly broad in all populations containing more than one specimen. Analysis of the different diploid populations revealed an embryonic/fetal phenotype that varied from the characteristically normal (Plate IA) as described by Mall (1918), Streeter (1920), Patten (1953), O'Rahilly (1967) and Moore (1981), to the mildly abnormal with focal anomalies to the markedly growth disorganized (GD_1-GD_4; Plate IC, Plate ID) as described by Poland and Miller (1973). Focal anomalies observed in the population of confirmed diploid fetuses also exhibit a range, including craniofacial, skeletal and truncal anomalies. It should be stressed that these were focal anomalies and that none of these fetuses were classifiable under any specific syndrome. These anomalies likely represent sporadic events which show the extent of phenotypic variation in diploid fetuses, which demonstrates that diploidy does not guarantee normal development.

Table 5.1: An Overview of Human Abortal Cases Employed in this Study

Genotype	Number	Karyotyped	Source
"Diploid"	349	No	TA/SA
46,XX/46,XY	27	Yes	TA
46,XX/46,XY	59	Yes	SA
46,XX/46,XY	12	Yes	SA
46,XX/46,XY	14	Yes	SA, PHM
46,XX/46,XY	6	Yes	SA, CHM
47,XY,+2	1	Yes	SA
47,XY,+4	1	Yes	SA
47,XX,+5	1	Yes	SA
47,XY,+5	1	Yes	SA
47,XX,+6	1	Yes	SA
47,XX,+7	1	Yes	SA
47,XY,+7	1	Yes	SA
47,XX,+8	2	Yes	SA
47,XY,+8	2	Yes	SA
47,XY,+9	1	Yes	SA
47,XY,+10	1	Yes	SA
47,XX,+13	2	Yes	SA
47,XY,+13	5	Yes	SA
47,XX,+14	3	Yes	SA
47,XX,+15	5	Yes	SA
47,XY,+15	6	Yes	SA
47,XX,+16	9	Yes	SA
47,XY,+16	6	Yes	SA
47,XX,+17	1	Yes	SA
47,XX,+18	4	Yes	SA
47,XY,+18	6	Yes	SA
47,XX,+19	1	Yes	SA
47,XY,+20	1	Yes	SA
47,XX,+21	21	Yes	SA
47,XY,+21	20	Yes	SA
47,XX,+22	2	Yes	SA
47,XY,+22	5	Yes	SA
45,XX,-21	1	Yes	SA
45,X	31	Yes	SA
3n	56	Yes	SA
4n	11	Yes	SA

Table 5.2: Compiled Phenotypes of Observed Diploid Cases

GENOTYPE	*N*	EMBRYO/FETUS		PLACENTA	
		EXTERNAL	INTERNAL/MICRO	GROSS	HISTOLOGIC
"Diploid" not karyotyped assumed to be diploid on the basis of structural normality	338	Normal development as described by Streeter (1920); Jackson (1909); O'Rahilly (1967); Patten (1953); Mall (1918)	no anomalies	Generally characterized by abundant growth activity in the first trimester: well vascularized chorial plate, dense placental development with vascularized closely packed excessively branched villi, gradual tapering of villi to the end of villous "tree", general villous structural uniformity	Well-formed chorial plate, closely packed irregular villi showing extensive branching with differentiation into conducting, intermediate and peripheral villi, presence of capillaries, arteries and veins, double layered trophoblastic epithelium, abundant sprout formation, and numerous trophoblastic islands
46,XX/ 46,XY TA	27	Normal development as described by Streeter (1920); Jackson (1909); O'Rahilly (1967); Patten (1953); Mall (1918)	NA (fragmented as a result of suction curettage)	Generally characterized by abundant growth activity in the first trimester: well vascularized chorial plate, dense placental development with vascularized closely packed excessively branched villi, gradual tapering of villi to the end of villous "tree", general villous structural uniformity	Well-formed chorial plate, closely packed irregular villi showing extensive branching with differentiation into conducting, intermediate and peripheral villi, presence of capillaries, arteries and veins, double layered trophoblastic epithelium, abundant sprout formation, and numerous trophoblastic islands
46,XX/ 46,XY SA	59	Empty sacs containing usually macerated GD_1-GD_4; GD_1 (19); Embryos - GD_2 (35; 2-6mm CRL); GD_3 (3; 15-17mm CRL); GD_4 (2; 11-12mm CRL); formed embryos - microcephaly, no ears, omphalocele	GD_2 - undifferentiated embryonic mass; poorly formed GD_3 - evidence of organogenesis; near normal histology GD_4 - marked autolysis; fragments of apparently normal skeleton and viscera	Characteristically thin, small loosely packed placentae with comparatively large chorionic sacs. GD_1 - clubbed, cystic, granular and/or focally agglutinated villi GD_2 - Breus mole, loosely packed placenta; clubbed, cystic, granular and/or focally agglutinated villi GD_3 - loosely packed placentae with fine filiform villi; no clubbing or cystic change GD_4 - small filiform villi showing no change, others which show minimal aggiutination with some clubbing and cystic change in villi	GD_1 - no NRC, hypovascular chorion, slit-like capillaries, compact and hydropic villi showing hypocellular stroma and hypoplastic trophoblast GD_2 - severely hypovascular and immature chorial plate, few NRC, loosely packed placenta, hypovascular compact and hydropic villi with hypocellular stroma and trophoblastic hypoplasia; ICC GD_3 - as in GD_2, but less severe GD_4 - as in GD_3, but less severe

46,XX/ 46,XY SA	12	generally abnormal craniofacies, absence of occipitofrontal bossing, hypertelorism, aural hypoplasia, DR, short limbs, sandal gap, rockerbottom feet, radial deviation of fingers, clinodactyly, .	urinary anomalies-enlarged kidneys, ascites, CH and generalized edema.	closely packed placenta	some intervillous fibrin deposition, hypovascular chorion and villi, mild villous branching abnormality, deep trophoblastic invaginations with secondary formation of intrastromal trophoblastic buds; one case of early amnion nodosum
46,XX/ 46,XY PHM	14	FCS (6), RCS without embryo (3); RCS with yolk sac and or embryo (2); ICS (1) **Embryos**: NE (1; 2mm) or Macerated (2; 12-18mm CRL) **No fetuses**	**Embryos**: marked growth disorganization with degenerative change associated with maceration	typically thin placentae with loosely packed villi which may show granularity, mild or extensive clubbing and early cyst formation	loose thin redundant amnion; immature hypovascular chorial plate with slit-like vessels containing NRC; small mixed compact and hydropic villi with variable stromal edema leading to cisterna formation; deep trophoblastic invagination leading to bud formation; general trophoblastic hypoplasia with focal hypoplasia of lacunar and syncytial trophoblast; reduced villus branching; ICC
46,XX/ 46,XY CHM	6	RCS without embryo, yolk sac, or cord;	NA	closely packed villi with clubbing and widespread cystic change and formation of grape-like vesicles(4-20mm)	absence of amnion; poorly developed inappropriate immature chorion; few slit-like capillaries without NRC; fewer larger mixed compact and hydropic/cystic villi showing reduced branching; inappropriate trophoblastic hyperplasia; focally prominent hyperchromatic extravillous extravascular trophoblast

Table 5.3: Compiled Phenotypes of Aneuploid Cases Examined in This Study

GENOTYPE	N	EMBRYO/FETUS		PLACENTA	
		EXTERNAL	INTERNAL/MICRO	GROSS	HISTOLOGIC
47,XY,+2 PHM	1	ICS-GD_2(1mm) with yolk sac	markedly abnormal GD embryo consisting of degenerate embryonic tissue	patchy clubbing of villi which are reduced in numbers leading to loose packing of the thin placenta; poorly vascularized chorial plate	poorly structured, immature hypovascular chorial plate; few NRC; combination of compact and hydropic villi; stromal hypocellularity with trophoblastic hypoplasia of villi; focal syncytial hyperplasia (PHM)
47,XY,+4	1	RCS-GD_1	NA	clubbed and cystic villi	thin, poorly structured avascular chorion; no NRC; reduced numbers of villi which show both microcystic and hydropic change; severely hypocellular and avascular villous patterns; trophoblastic hypoplasia
47,XX,+5 47,XY,+5	2	FCS;ICS-GD_2(2mm)	NA-lost in processing	thin, loosely packed placenta, hypovascular chorial plate and villi, peripheral clubbing	range of immature to well-developed chorial plate containing either a few slit-like capillaries or abundant development of chorial vasculature, some NRC's, villi are either normal or show hypocellular stroma with intrastromal trophoblastic buds
47,XX,+6	1	FCS-GD_1	NA	avascular chorial plate overlying a thin, poorly structured placenta; villi are scanty and ill-defined	immature, avascular chorion; no NRC; avascular villi exhibiting stromal hypocellularity and severe trophoblastic hypoplasia
47,XX,+7 47,XX,+7 PHM	2	RCS; ICS-GD_3: abnormal craniofacies (no true formation of any facial structures) coloboma, microcephaly, ectopia cordis, agenitalia, hypoplastic limbs	NA	pale, translucent hypovascular chorial plate; thin, loosely packed placenta; hypovascular villi showing clubbing, cyst formation, granularity	immature chorion containing slit-like capillaries; few NRC's; reduced number and distribution of mixed compact and hydropic, hypovascular villi; cystic formation; intrastromal trophoblastic buds;

47,XX,+8 (1) 47,XY,+8 (2)	3	FCS; RCS-GD_2	NA	hypovascular, loosely packed chorionic villi showing clubbing and/or cystic change	immature hypovascular chorial plate; with or without NRC; redundant amnion; reduced numbers of irregular and loosely packed villi of mixed compact and hydropic patterns; intravillous stromal hypocellularity; trophoblastic hypoplasia;
PHM 47,XX,+8	1	RCS	NA	loosely packed villi with widespread clubbing and patchy microcystic change	abundant, redundant amnion; NRC; reduced numbers of irregular loosely packed villi showing a mixed compact and hydropic pattern; stromal hypocellularity; excess of syncytial sprouts
47,XX,+9	1	ruptured sac without embryo	NA	thin placenta; loosely packed, fine filiform chorionic villi	hypovascular chorial plate and villi; some NRC's; mixed compact and hydropic villi; villous stromal and trophoblastic hypoplasia
47,XX,+10	1	RCS	NA	pale, translucent, severely hypovascular chorial plate; loosely packed focally clubbed villi with patchy agglutination	poorly developed, severely hypovascular chorion; NRC; virtually avascular mixed, compact and hydropic villi; trophoblastic hypoplasia; small, rounded villi with little branching or buckling of coastlines
47,XX,+13 (2) 47,XY,+13 (5)	3	ICS-GD1; RCS-GD2; FCS	NA	thin placenta; range of loosely or closely packed villi; either focally clubbed, filiform, or hydropic	poorly structured avascular chorion; no amnion/yolk sac or NRC in GD_1; avascular villi with reduced branching showing a mixed compact and hydropic pattern; stromal hypocellularity and trophoblastic hypoplasia
	7	**Fetuses**: holoprosencephaly, diastasis recti, hypertelorism, lowset ears, short limbs, polydactyly.	bilobular right lung, renal agenesis (right side).	loosely packed hypoplastic placenta with finely granular villi; thin, translucent hypovascular chorionic plate; clubbed or hydropic villi	thin amnion; hypovascular chorion with slit-like capillaries; NRC; reduced villous branching; villous hypovascularity; trophoblastic hypoplasia
47,XX,+14 47,XY,+14	3	FCS; RCS with GD_4 embryo (twelve, 20mm CRL)-craniofacial abnormality, i.e., coloboma, microcephaly	NA	pale, translucent chorial plate; Breus mole; loosely packed placenta to abundant, closely packed placenta with focal agglutination of villi	hypoplastic amnion and chorion, markedly immature hypovascular chorion (with NRC's); few small hypovascular villi showing focal stromal edema and trophoblastic hypoplasia

47,XX,+15 (4) 47,XY,+15 (6)	10	RCS (5); RCS with GD_2 (3); ICS (2)	poorly developed embryonic tissue	generalized hypoplasia, loss of structural uniformity, and disturbed morphogenesis with mixtures of compact and clubbed or cystic villi. -small sac; hypo- or avascular chorion; thin placenta with reduced linear growth and branching; mixture of thin and compact, clubbed or cystic villi	hypoplastic, hypocellular, immature chorion with absent or slit-like villi; sparse NRC's; loosely packed, mostly rounded villi with poor branching and differentiation; hypocellular stroma with variable matrix ranging from normal to hydropic to cystic; generalized trophoblastic hypoplasia; scattered intrastromal trophoblastic buds; ICC
PHM 47,XX,+15	1	RCS	NA	closely-packed villi with mild focal clubbing	thin amnion; thin, immature hypovascular chorion with slit-like capillaries; NRC; abundant villi with mixed compact and hydropic structures; mostly round villi, little buckling with trophoblastic bud formation; focal persistence of lacunar trophoblast; syncytial knot prominence
47,XX,+16 (7) 47,XY,+16 (5)	12	FCS; RCS; ICS- GD_1, GD_2(1mm)	NA: embryos lost in processing	Breus mole(1); range in chorial plate of well-vascular to hypovascular; loosely packed, poorly structured placentae; villi may show clubbing, cystic change and granularity	avascular or hypovascular chorion; loose placenta with reduced numbers and branching of villi in most placentae; mixtures of hypovascular, predominantly compact, and hydropic round villi, stromal hypocellularity; occasional intrastromal trophoblastic bud; generalized trophoblastic hypoplasia; ICC
PHM 47,XX,+16 (2) 47,XY,+16 (1)	3	RCS; ICS	NA	thin loosely packed placenta without recognizable vessels; hypovascular villi with focal clubbing	poorly structured severely hypovascular chorial plate; slit-like capillaries with NRC; ill-defined abnormally structured yolk sac; loosely packed villi with reduced differentiation; mixed hydropic and compact, hypovascular and hypocellular villi; ICC; focal excess of syncytial sprouts; focal prominence of lacunar trophoblast
47,XX,+17	1	RCS	NA	hypovascular chorial plate; thin placenta; loosely packed, focally clubbed villi	immature, severely hypovascular chorion with slit-like capillaries; NRC's present; mostly round, mixed compact and hydropic villi which show severe hypovascularity and variable degrees of stromal hypocellularity and trophoblastic hypoplasia

47,XX,+18 (3) 47,XY,+18 (6)	1	FCS-GD$_1$	NA	thin placenta; small loosely packed villi with moderate clubbing	thin, poorly structured avascular chorion; no NRC; scanty, loosely packed, poorly developed villi showing mixed hydropic and compact pattern; avascular villi with stromal hypocellularity; scattered clusters of coarsely granular Hofbauer cells; trophoblastic hypoplasia

47,XX,+18 (3) 47,XY,+18 (6)	9	Fetuses: small mouth, small chin, microcephaly, flat occiput, low set malformed ears, hypognathism, peaked-bird-like craniofacies, iniencephaly associated with complete rachischisis, hypotelorism, protuberant tongue, acalvaria, short flat neck, cystic hygroma, long neck, short limbs, long fingers, equinovarus deformity, rockerbottom deformity, syndactyly, clinodactyly, diastasis recti, omphalocele, imperforate anus, ambiguous genitalia, agenitalia, general edema	VSD, agenesis of corpus callosum, vaginourethral convergence, duodenal stenosis	range from normal placenta with well-formed abundant villi to villous hypoplasia accompanied by poorly vascularized chorial plate with mild focal villous clubbing	range from abundant well-formed chorion, amnion and villi to well-formed villi accompanied by a hypovascular, bloodless chorion, to villi which lack structural uniformity; focal large irregularly branched, hypocellular, hypovascular villi; mixtures of compact and hydropic villi
PHM 47,XX,+18 (1)	1	RCS	fragments of severely growth disorganized embryo	abundant, closely packed, finely granular villi with some early clubbing but no cystic change	poorly structured hypovascular chorion; NRC; excess syncytiotrophoblast; irregular, compact and hydropic hypovascular villi; ICC
47,XX,+19	1	RCS-GD_2 (3mm)	severely growth disorganized embryo with formation of periderm and complete absence of organization of the undifferentiated embryonic cells	Breus mole; loosely packed chorionic villi; peripheral clubbing of mostly round villi	thin hypovascular chorion; NRC's present; fewer, less uniform hypovascular villi; hypocellular stromal matrix; trophoblastic hypoplasia; mixed compact and hydropic villi; ICC

47,XY,+20	1	FCS-GD_1	NA	loosely packed granular, focally clubbed villi, avascular chorial plate	hypovascular chorial plate with slit-like capillaries, no NRC, mixtures of compact and hydropic, hypovascular villi with stromal hypocellularity and trophoblastic hypoplasia
47,XX,+21 (20) 47,XY,+21 (20)	40	**GD embryos:** FCS; RCS; GD_1; GD_2 (5mm) with cord and yolk sac	NA severely growth disorganized degenerate embryo	**Embryos:** - thin, hypovascular chorion; loosely packed, extensively clubbed, mildly cystic rounded villi; focal granularity or agglutination	**Embryos:**- redundant, thin, hypocellular amnion; poorly structured hypovascular hypocellular chorion; NRC except in GD_1; fair number of closely packed villi showing reduced branching and linear growth; round severely hypovascular villi with slit-like capillaries; mostly compact pattern; stromal hypocellularity; scattered clusters of Hofbauer cells
47,XX,+21 (20) 47,XY,+21 (20)		**Non-GD Embryos:** macerated embryos (fragments and complete at 26mm CRL); apparently normal fragmented embryonic tissue	**Non-GD Embryos:** marked autolysis, no obvious malformation	**Non-GD Embryos:** large sac; thin placenta; straight filiform, loosely packed to abundant villi with patchy clubbing, but no cyst formation; focal granularity	**Non-GD Embryos:** poorly structured immature chorion; NRC; scanty mostly round and straight villi with reduced branching; severe villous hypovascularity, stromal hypocellularity; patchy edema; hypoplastic villous trophoblast; persistence of immature lacunar trophoblast
47,XX,+21 (20) 47,XY,+21 (20)		**Fetuses:** hypertelorism, mongoloid slanting of the orbits, flat occiput, aural anomalies, microstomia, short neck, excess nuchal skin, short upper limbs, short middle phalanx on fifth finger, clinodactyly, rockerbottom deformity, diastasis recti, cutis navel, simian crease, sandal gap, hydrops, cystic hygroma, omphalocele, and platysma	**Fetuses:** ASD, duodenal stenosis, incomplete liver lobulation, VSD, pleural effusion, ascites, atrio-ventricularis communis	**Fetuses:** - range from no obvious abnormality in a fairly abundant, closely packed, well-vascularized placenta to slightly granular, loosely packed, poorly differentiated villi; one case of Breus mole	**Fetuses:** - hypovascular chorial plates; abundant irregular villi which are mostly compact; deep trophoblastic invaginations culminating in the formation of intrastromal trophoblastic buds; abnormal branching of rather large hypovascular, hydropic villi; ICC

PHM 47,XX,+21	1	RCS; degenerated, macerated embryonic tissue with differentiation of cranial pole and at least one limb bud	definite embryonic organization; evidence of organogenesis	thin hypovascular chorion; thin, poorly structured placenta; closely packed hypovascular villi with focal clubbing but no microcyst formation	thin amnion; hypovascular chorion with slit-like capillaries; NRC; mixed compact and hydropic, straight villi with reduced branching; general trophoblastic hypoplasia; persistence of lacunar trophoblast; excess syncytial trophoblast; ICC
47,XX,+22 (2) 47,XY,+22 (5)	6	**GD embryos:** RCS-GD_1; ICS with GD_2 (3mm)	autolyzed, undifferentiated and unorganized tissue	thin placenta; loosely packed; pale, hypo- or avascular chorial plate with filiform or focally clubbed villi	hypovascular chorion and villi with formation of scanty irregularly dispersed slit-like simple blood vessels without maturation into arteries and veins; generalized hypoplasia reducing linear growth and branching of fewer loosely packed villi; predominance of undersized villi; severely hydropic and compact villi; intrastromal trophoblastic buds
	1	**Non-GD embryo:** malformed embryo, macerated embryo - low cranial vault, absence of nasal process, aural anomalies, short limbs, agenitalia, no anus	**Non-GD embryo:** boot-shaped heart; bilateral absence of diaphragm and pericardium; lowset kidneys; absence of bladder, large bowel and anal canal	**Non-GD embryo:** abundant, closely packed, well-vascularized villi without cystic change or clubbing	**Non-GD embryo:** firmly adherent amnion; poorly structured severely hypovascular chorion; severely hypovascular mixed hydropic and compact villi; trophoblastic hypoplasia; intrastromal trophoblastic buds; irregular villous branching
45,XX,-21 PHM	1	RCS	NA	pale translucent avascular chorial plate; thin placenta (5mm); loosely arranged fine, filiform villi with focal clubbing and granularity	thin amnion; poorly structured hypovascular chorial plate; NRC; mixed hydropic and compact pattern; excess syncytiotrophoblast; immature lacunar trophoblast
45,X	18	**GD embryos:** FCS (3), RCS without embryo (2) GD_2 - FCS (1); RCS (9); ICS (3); nodular embryo (2-4mm)	**GD embryos:** marked growth disorganization, undifferentiated tissue	**GD embryos:** Breus mole; large sac; pale, translucent chorial plate, thin placenta; scanty villi with or without clubbing or cystic change; focal agglutination	**GD embryos:** redundant, loosely adherent immature amnion; immature hypovascular chorion; NRC; reduced branching of mostly compact villi; hypoplastic trophoblast; focal, free-floating sprouts; range of good or reduced differentiation of villi; irregular coastline; stromal hypocellularity; possible edema

45,X	3	**Non-GD embryos:** formed embryos (10-22mm CRL); pigmented eyes; marked distortion (retention associated)	**Non-GD embryos:** macerated internal viscera; limited critical evaluation	**Non-GD embryos:** thin chorial plate and placenta; poorly-defined blood vessels; loosely-packed filiform villi without cystic change	**Non-GD embryos:** fibrotic amnion; poorly structured, immature, severely hypovascular chorion with slit-like capillaries containing NRC; loosely-packed villi with reduced linear and radial growth; trophoblastic hypoplasia, small round villi with dense hypovascular core; focal prominence of free-floating, compact, syncytial sprouts; minimal patchy stromal hydrops
45,X	8	**Fetuses:** generalized edema, mongoloid slanting of eyes, prominent supraorbital ridges, hypertelorism, peaked triangular face, posterior rotation of ears, lowset and long ears, thin aural helix, reduced occipitofrontal bossing, persistent metopic suture, cystic hygroma, short neck, short thorax, short limbs, clinodactyly, abdominal bulging, omphalocele	**Fetuses:** pleural effusion, ascites, pericardial effusion, tubular hypoplasia of the aortic isthmus, coarctation of the preductal aortic arch, short aortic arch, single ventricle, microcardia, horseshoe kidney, short intestine, slit-like anus, ovarian hypoplasia	**Fetuses:** loose, translucent amnion; chorial plate with vascular hypertrophy; thin placenta; Breus mole; abundant to loosely packed granular villi without clubbing or cystic change	**Fetuses:** chorial plate well preserved and well vascularized; placental hypoplasia with reduced numbers of intermediate and peripheral villi showing stromal hydrops and hypovascularity; patchy prominence of small, shrunken pyknotic syncytial cells; irregular villous branching; focal enlargement of the intervillous space; occasional villous has regressive calcifying trophoblastic invagination; villous fibrosis and reduced linear and radial growth
45,X PHM	2	FCS-brownish mass of embryonic tissue (3mm); RCS (23mm CRL embryo with pigmented eye and short paddle-shaped limbs)	NA	thin, hypovascular chorial plate; thin placenta; well-packed villi with clubbing and granularity	thin, redundant amnion, hypovascular chorion; NRC; variable density of placenta; some hydropic and compact villi; trophoblastic hypoplasia; hydropic stroma; syncytial hyperplasia; irregular coastlines

Table 5.4: Compiled Phenotypes of Polyploid Cases Examined in This Study

GENOTYPE	*N*	EMBRYO/FETUS		PLACENTA	
		EXTERNAL	INTERNAL/MICRO	GROSS	HISTOLOGIC
3n Non-molar	16	**GD embryos:** RCS; FCS; ICS-GD_2(2mm)	**GD embryos:** growth retarded, undifferentiated cellular mass	**GD embryos:** Breus mole; thin placenta with loosely packed villi and poorly vascularized chorial plate	**GD embryos:** amnion focally adherent to hypovascular chorion; NRC; compact villi with irregular coastline; multiple deep trophoblastic invaginations and hyperchromatic trophoblastic buds
3n Non-molar	2	**Non-GD embryos:** formed embryos(8-10mm) apparently normal embryonic fragments; abnormal embryos with flat cranial vault, severe microcephaly, undifferentiated facial structures, absence of lower limbs, short trunk	**Non-GD embryos:** marked autolysis	**Non-GD embryos:** thin placenta; scanty to abundant, pale, focally agglutinated villi with or without clubbing, cyst formation	**Non-GD embryos:** thin amnion; immature poorly structured hypovascular chorion; NRC; reduced numbers of villi with reduced branching and differentiation; scattered hyperchromatic stromal cells; compact and hydropic villi with irregular coastlines
3n Non-molar	9	**Fetuses:** thin gracile fetus, mild retrognathism, syndactyly, genital hypoplasia, hydrocephaly, mandibular hypoplasia	**Fetuses:** VSD	**Fetuses:** thin placenta with loosely packed villi and well-vascularized chorial plate	**Fetuses:** thin amnion; immature poorly structured chorion; reduced linear growth and branching of rounded villi; apparent reduction in the number of intermediate villi with a predominance of small peripheral structures; irregular coastlines and intrastromal trophoblastic buds

3n PHM	29	FCS (19); RCS without embryo (1); embryonic fragments (1); fetal fragments (2); GD_1 (1); abnormal embryos (3); fetuses (3) Abnormal Embryos (10-14mm CRL): microcephaly, no eyelids, complete failure of development of central facial structures and ears, short trunk, growth retarded Fetuses: short neck, short limbs, protuberant abdomen, round cranium, poorly-defined philtrum, syndactyly, sandal gap	**Embryos**: too autolyzed for meaningful evaluation; weak attempt at organogenesis **Fetuses:** ASD, small thorax, absence of uterus and fallopian tubes in one female fetus; fused uterus; fetuses (2) too autolyzed for critical evaluation	pale, hypovascular chorionic villi with peripheral clubbing and cystic development	thin amnion; thin hypovascular chorion; loose villous arrangement with reduced numbers and predominantly conducting and intermediate structures over small peripheral villi; compact and hydropic villi with focal pooling of edema but no cysts; deep trophoblastic invaginations and intrastromal trophoblastic buds; focal inappropriate trophoblastic hyperplasia; frankly cystic villi
4n Non-molar	9	RCS; RCS-GD_1	NA	Non-molar - thin poorly vascularized chorion; abundant closely packed villi which may show clubbing, cyst formation, agglutination, or granular change; Breus mole	thin, avascular chorial plate; hypoplastic amnion; large hydropic, avascular villi exhibiting variable stromal hypocellularity and trophoblastic hypoplasia; mostly round, compact villi with reduced branching and differentiation; focal prominence of free-floating syncytial sprouts
4n PHM	2	FCS	NA	Molar - thin placenta with hypovascular chorion; loosely packed villi showing focal or extensive clubbing, granular or cystic change	thin avascular or hypovascular chorion; no amnion detected; no NRC; avascular, hypocellular hydropic edematous; a mixture of compact and predominantly hydropic or cystic villi; occasional ICC; patchy inappropriate excess of trophoblast especially syncytial trophoblast; focal granular Hofbauer cells

Similarly, the placental phenotypic range varied usually exhibiting normal development marked by thick placentae (Plate IVA, B) with abundant villus growth grossly, and well vascularized chorion and villi histologically placentae (Plate VIA, B). Cases of structurally abnormal development were marked by mild to severe placental hypoplasia corresponding to poor amniotic and chorionic (Plate IVC) development, reduced chorionic and villous vascularity, and reduced villus branching and differentiation. Complete hydatidiform moles were also found which were distinguished from structurally abnormal conceptuses by the presence of large hydropic and cystic villi showing patchy inappropriate trophoblastic hyperplasia involving the cytotrophblastic and the syncytiotrophoblast.

The relationship between embryo/fetal development and that of the placenta is a tight one. Generally, normal embryo/fetal development was associated with normal placental development. The fetuses with focal anomalies tended to show a mild degree of placental anomaly affecting the chorionic and villus vasculature, and villus branching. In contrast, the diploid growth disorganized embryos showed a more severe expression of placental hypoplasia; thin small loosely packed placentae with chorionic and villus hypoplasia and possible clubbing, cystic change, granularity, or agglutination which worsened as the embryonic phenotype approximated GD_1 development. The placental phenotype of partial hydatidiform mole paralleled that of the growth disorganized classes, but showed focal hyperplasia of the syncytiotrophoblast; embryonic development was generally poor. Only the complete hydatidiform moles showed poor placental development associated with trophoblastic hyperplasia and the consistent absence of embryonic formation. Thus, it is apparent that poor embryonic development correlates strongly with poor placental development in diploidy. This will be a recurrent theme with all genotypic populations.

B Aneuploidies

B.1 Non-viable Trisomy

The non-viable trisomies (2, 4, 5, 6, 7, 8, 9, 10, 14, 15, 16, 17, 19, and 20) showed generally similar phenotypes. Embryonic development was arrested in the very early stages at periods as outlined by Boué and Boué (1985). Consequently, the variation in embryonic development was restricted to different classes of growth disorganization ranging from GD_1 to GD_4 and included cases of fragmented and ruptured chorionic sacs without identifiable embryos with or without NRC, demonstrating the ability to reach at least the 3^{rd}-4^{th} week of development.

All descriptions of these conceptuses are in greater detail than that found in the literature. The new findings include the following at the embryonic level:

1) The trisomy 7 GD_4 embryo which was only examined at the externally due to maceration demonstrated failure of formation of facial structures, microcephaly, ectopia cordis, hypoplastic limbs and agenitalia.

2) The trisomy 22 embryo (Plate IB) was a unique case that showed complete closure of the neuropores with failure of external caudal development involving the anus and genitalia, and the absence of the diaphragm, pericardium, bladder, internal genitalia and anal canal. The phenotype of this case closely paralleled that of a liveborn trisomy 22 conceptus (Kukolich, 1989).

At the placental level, the study has contributed:

1) the complete descriptions of placental phenotypes of trisomies 2, 4, 5, 6, 7, 8, 9, 10, 13, 14, 15, 16, 17, 18, 19, 20, 21, and 22 (Plate VB), (Table 5.3). This is the first systematic description of placentae with these specific forms of trisomy.

2) the first documented descriptions of trisomies 6 and 19

3) Partial hydatidiform mole in monosomy 21 never recorded before.

Placental development of this range of trisomies demonstrated phenotypic variability although a certain triad of features was usually present (Plate IVD, VA; Plates VIC, D; Plate VIIB). These involve generalized hypoplasia, loss of villous structural uniformity and disturbed morphogenesis with mixtures of compact and clubbed or cystic villi. Grossly, the placentae showed hypo- or avascular chorial plates, amniotic hypoplasia, and villous changes which may include clubbing, cyst formation, hydrops, granularity and agglutination. Histology showed stromal hypocellularity and trophoblastic hypoplasia with intravillous cytotrophoblastic cells, which are isolated clusters of trophoblastic cells found within the villous core but outside of the trophoblastic basement membrane; intravillous cytotrophoblastic cells are not diagnostic of trisomy as they are both non-specific, occurring in cases of polyploidy, and are not always present in trisomy.

Branching was reduced and accompanied by a lack of villous differentiation with preferential increases in numbers of large intermediate and conducting over development of small peripheral villi. Erratic formation of intrastromal trophoblastic buds (Plate VIIC) resulting from deep trophoblastic invaginations were observed in trisomies 5, 7, 15, 16, and 22; these are quite rare in normal development (observed in only one of the diploid cases in this series), but are usually found in triploidy (Honoré *et al.*, 1976; 1989).

4) Furthermore, trisomies 2, 7, 8, and 15 also demonstrated partial mole formation in some cases which were mostly recovered as fragmented, ruptured or empty intact chorionic sacs and rarely as GD_1 or GD_2 (1-2mm) embryos. The placentae were thin with hypovascular chorial plates and hypovascular clubbed villi. Microscopically, these findings were confirmed, combinations of compact and hydropic villi with stromal hypocellularity and trophoblastic hypoplasia were identified which was accompanied by focal inappropriate syncytiotrophoblastic hyperplasia and the presence of intravillous cytotrophoblastic cells in trisomies 15 and 16.

B.2.1 Potentially Viable Trisomies: E/F.

The viable trisomies 13, 18, and 21 demonstrated characteristic phenotypes. The new findings include:

1) diastasis recti in trisomy 13, 18 and 21 (Plate IIB).
2) bilobed right lung in trisomy 13
3) iniencephaly with complete rachischisis and agenesis of the corpus callosum and olfactory bulbs, (although agenesis of the olfactory bulbs is not, itself, a new finding) in trisomy 18
4) agenitalia in trisomy 18
5) duodenal stenosis in trisomy 18
6) cutis navel in trisomy 21
7) presence of platysma in trisomy 21.

B.2.2 Potentially Viable Trisomies: Placenta

The placentae of trisomy 13 exhibit a wide range of phenotypes which tended to correspond closely to the advanced stage of embryonic development. Although the placentae usually showed the same generalized features of trisomy, certain trends were observed. Marked differences in placental quality were observed histologically with the trisomy 13 growth disorganized conceptus; this placenta exhibited severe hypovascularity that may be tightly associated with the poor outcome of this pregnancy. Other cases showed moderate hypovascularity of the chorial plate and focally clubbed or hydropic villi grossly and microscopically.

The placental phenotype in trisomy 18 ranges grossly from the apparently normal to that with villous hypoplasia and mild focal clubbing. The histologic presentation closely parallels this; the range extends from abundant well-formed placentae with hypovascular chorial plates and villi to placentae with mixtures of compact and hydropic villi which lack structural uniformity and show focal large, irregularly branched, hypocellular and hypovascular development which is typical of trisomy. This phenotypic range is expected, as suggested by Kalousek *et al.* (1989). Kalousek suggested that those trisomy 18 conceptuses which spontaneously abort do so, because they are pure trisomy 18 conceptuses. They suggest that placental function drives intrauterine survival and consequently, the survival of trisomy 18 conceptuses to term is dependent on placental quality approximating normal; for this to happen, a second diploid line must be present. However, the cases of near-normal placental development in trisomy 18 were therapeutically aborted as a result of cytogenetic analyses from chorionic villus sampling or the chorial plate, and did not demonstrate mosaicism. These cases of trisomy 18 indicate that mosaicism is not necessary for near-normal placental development, rather near normality is part of the placental phenotypic spectrum in trisomy 18.

A similar placental spectrum was found in trisomy 21. Cases where embryonic development arrested early, that is any time before 6 weeks DA, showed different

placental morphology than conceptuses with fetogenesis. Early developmental arrest, as with GD showed gross features of a thin hypovascular chorion and either abundant closely packed or thin loosely packed round villi which exhibited agglutination, clubbing or cystic change. Histologic assessment supported the gross observations and showed a redundant, hypocellular amnion, scattered clusters of Hofbauer cells and other generalized features of trisomy.

Advanced embryonic development correlated with improved placental morphology, although fetal cases were exhibiting much milder, subtle changes. Grossly some placentae showed near normal morphology. Others had granular texture and were loosely packed. One case of Breus mole was also recorded. Histologically, however, these cases also showed the triad of findings normally associated with trisomic placentae.

As with the non-viable trisomies, partial mole formation was associated with this group of trisomies, specifically trisomies 18 and 21. Although the trisomy 18 partial hydatidiform mole was recovered as a ruptured chorionic sac, the trisomy 21 conceptus contained a fragment of degenerate macerated embryonic tissue showing differentiation of the cranial pole and at least one limb bud. The placental findings in the two cases were fairly similar, demonstrating a thin hypovascular chorion, and a poorly structured placenta with closely packed, hypovascular, focally clubbed villi without cystic change. Microscopically, these findings were confirmed as was the presence of intravillous cytotrophoblastic cells and inappropriate focal syncytiotrophoblastic hyperplasia.

B.3 Monosomy

One case of autosomal monosomy was recorded (monosomy 21) which was a partial hydatidiform mole recovered as an ruptured chorionic sac. The placenta was thin with a pale, translucent avascular chorial plate. The fine, filiform villi were loosely arranged with focal clubbing and granularity. Microscopically, the thin amnion was adherent to poorly structured severely hypovascular chorial plate. The slit-like chorial vessels contained fetal nucleated red cells and the villi were of a mixed compact and hydropic or cystic pattern. Excessive syncytial trophoblast and immature lacunar trophoblast was also identified.

Like the previously discussed aneuploidies, the 45,X cases showed a range of phenotypes. Embryonic development either arrested at or before the 4th week (nodular embryos, GD_2) or continued to yield formed embryos and fetuses (Plate IIA). In other words, ruptured chorionic sacs with or without nucleated red blood cells, GD_2 nodular embryos, and formed embryo/fetus were all recovered. Histology of the GD_2 embryos revealed growth disorganization and undifferentiated embryonic tissue. The formed, macerated embryos showed poor definition of the facial structures; histology identified the formation of internal viscera, although they were markedly macerated. The formed embryos were markedly macerated. The external and internal features of formed fetuses confirmed, but did not expand on previous findings (Table 5.4).

The placentae of 45,X conceptuses developed in a manner which correlated tightly with the degree of embryonic expression. The GD conceptuses had redundant, loosely adherent amnion, immature hypovascular chorion, reduced branching of villi and a tendency toward excessive free-floating syncytial sprouts. In contrast, the placentae of 45,X fetuses were of variable thickness with abundant, closely packed filiform, granular villi. Histologically, the chorial plate was well-developed with good vasculature. Placental hypoplasia was demonstrated by small immature, mostly conducting villi (Plate VIIA) showing reduced linear and radial growth, and stromal hypocellularity and focal hydrops.

Two cases of 45,X partial hydatidiform moles were also recorded showing variable degrees of embryonic development. One case contained only a small fragment of autolyzed embryonic tissue whereas the other showed comparatively advanced development with an embryo (23mm CRL). The embryo had a fragmented face with pigmented eyes and short paddle shaped limbs demonstrating growth retardation, no other anomalies were identified. In both cases, a thin placenta was found. The chorial plate was hypovascular and the well-packed villi showed clubbing and granularity. These placentae demonstrated thin amnions, hypovascular chorions, nucleated red blood cells and mostly rounded compact, hydropic and cystic villi with irregular coastlines and extreme syncytiotrophoblastic hyperplasia.

C Polyploidy (Table 5.4)

The triploid conceptuses were either molar or non-molar based on placental morphology. Non-molar fetuses had obvious focal anomalies. A ventral septal defect, bilobed right lung, lowset kidneys, and adrenal hypoplasia were recorded as the only detectable internal anomalies.

The gross examination of the non-molar placentae showed marked villous hypoplasia although the chorial plate did not appear to be affected. Microscopy revealed amniotic and chorionic hypoplasia, with reduced villous linear growth and branching. The villi also demonstrated irregular coastlines with deep intrastromal trophoblastic buds.

The molar triploids (partial hydatidiform moles) demonstrated a range of developmental arrest occurring in either early embryogenesis or in fetogenesis. These conceptuses were either ruptured chorionic sacs, GD_2 embryos, or embryos and early fetuses with focal anomalies (Plate IIC). The latter exhibited micro- or hydrocephaly and failure of development of the central facial structures. Fetal anomalies included an atrial septal defect, small thorax and absence of uterus and fallopian tubes. This is the first time failure of internal genitalia formation has been documented in a molar triploid fetus.

Grossly, triploid molar placenta shows hypovascular chorionic villi with clubbing and cystic change (Plate VC). Microscopically, amniotic and chorionic hypoplasia is evident. Again, trophoblastic anomalies are present which manifest themselves as hyperchromasia, intrastromal buds and syncytial hyperplasia, confirming previous descriptions (Honoré *et al.*, 1976; Szumlan *et al.*, 1978a,b).

The molar and non-molar tetraploid (Table 5.4) conceptus appears to arrest in embryonic development very early. Where tissue was recovered, embryonic development arrested by the third week of development (GD_1). This finding supports the earlier documented description (Boué and Boué, 1985).

The molar placenta was loose and thin with a hypovascular chorion and granular villi exhibiting clubbing and cyst formation. Histology revealed occasionally avascular chorial plates, absence of amnion which may be artifactual, and mostly avascular hypocellular hydropic villi. There was also an excess of mature syncytial sprouts, the rare presence of intrastromal trophoblastic buds and focal inappropriate hyperplasia of syncytial trophoblast. In comparison, the non-molar tetraploid placenta demonstrated a variable though mostly abundant villus pattern on a generally hypovascular chorion. One case of Breus mole was recorded. These findings were confirmed histologically, and mostly round small compact villi were also detected as was the focal prominence of free-floating syncytial sprouts and hyperchromasia of trophoblastic and stromal nuclei.

In summary, irrespective of genotype placental hypoplasia shows a strong positive correlation to embryonic anomaly. While numerous focal embryonic and fetal anomalies were identified in this study, the placenta showed a range of abnormality affecting each placental structure:

1) Amnion - normal to hypoplastic (thin) to absent.
2) Chorion - normal well-vascularized and well-structured to thin and hypovascular with primitive slit-like capillaries and poor, if any, arterial/venous formation.
3) Villi - generally hypo- or avascular. They may also be clubbed, cystic or granular. Microscopically, loose hypocellular stroma is identified. Typically, trophoblastic disturbances included hypoplasia, intrastromal bud formation resulting from deep trophoblastic invaginations, or intravillous cytotrophoblastic cells.

PLATE I

A. Normal embryo at 7 weeks DA.

B. Trisomy 22 macerated, disrupted embryo of approximately 7 weeks DA showing marked microcephaly, a wide rima oris (arrow), absence of nose and ears, short paddle shaped limbs and absent genitalia.

C. Chorionic sac with a growth disorganized nodular embryo(arrow).

D. Histology of growth disorganized (GD_2) embryo showing lack of organization of embryonic tissue.

PLATE I

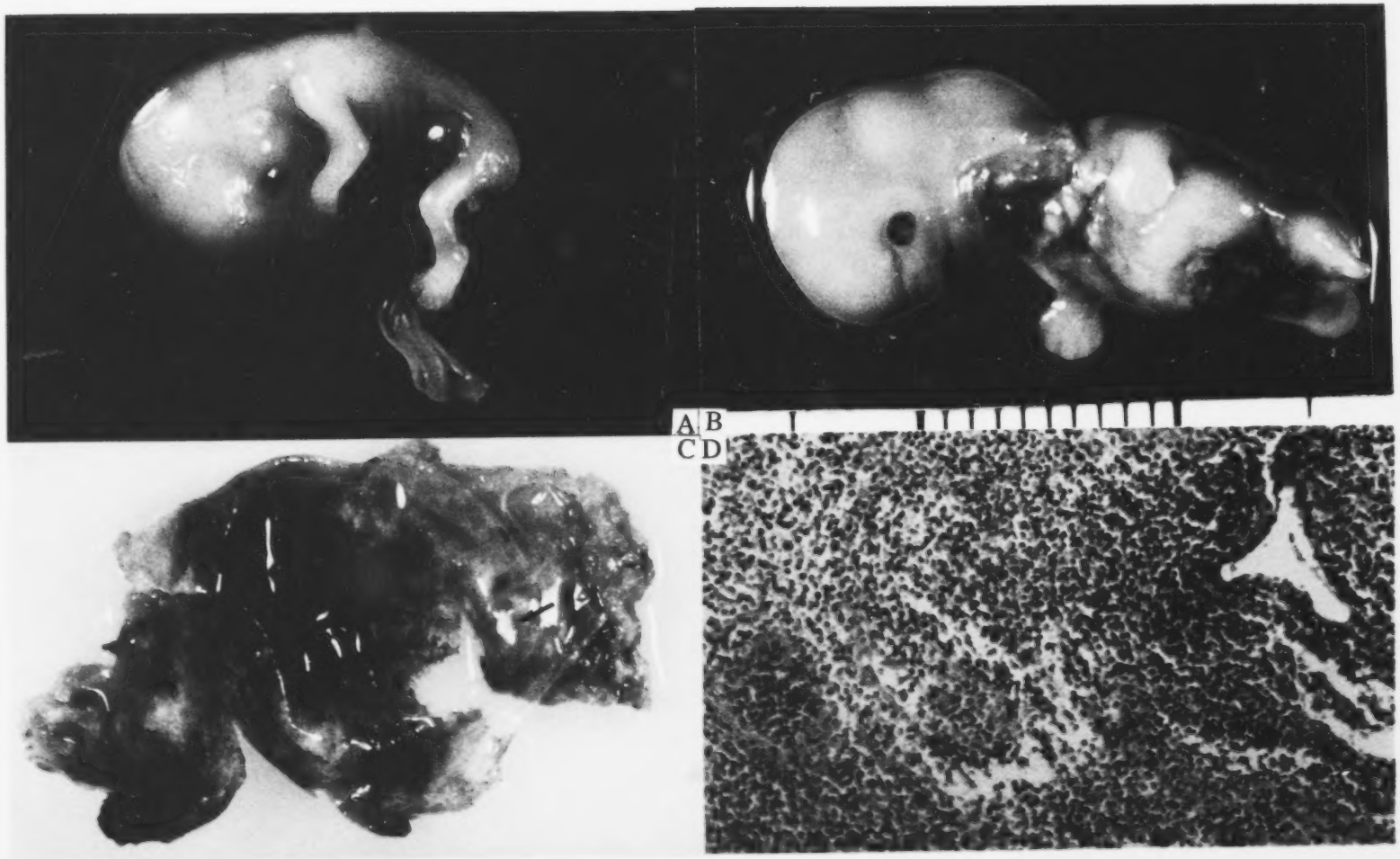


PLATE II

A. Monosomy X female fetus with generalized hydrops and nuchal cystic hygroma.
B. Trisomy 21 fetus with diastasis recti (arrow) and short upper limbs.
C. Triploid fetus (69,XXX) from a PHM showing medial cleft lip and diastasis recti (arrow).

PLATE II

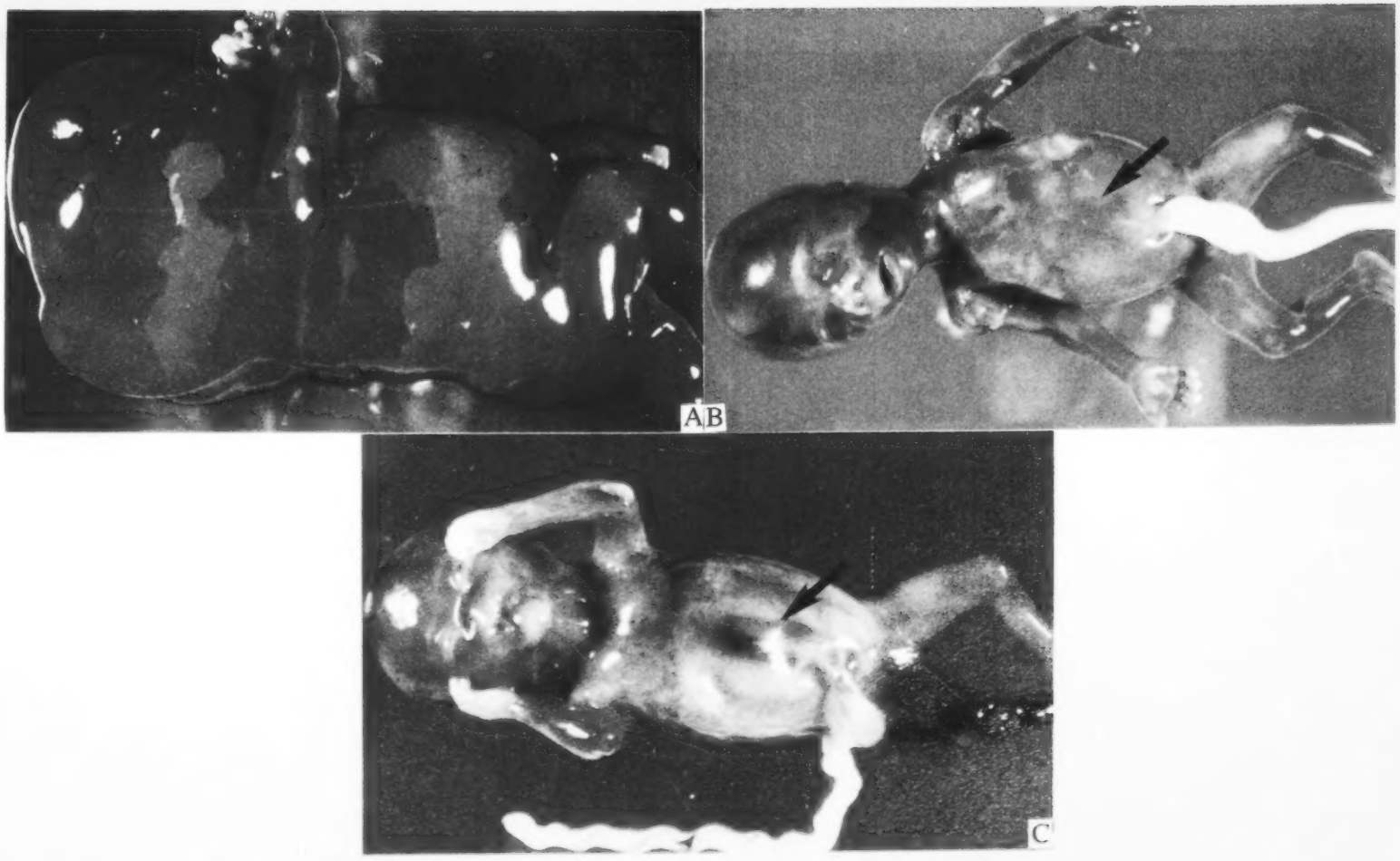


PLATE III

A. Normal craniofacies in a fresh, congested fetus.
B. Bilateral cleft lip in a trisomy 18 fetus.
C. Two cases of trisomy 21 showing marked differences in phenotype. The fetus on the left has a large round head, a wide BPD and face. The fetus on the right is hyperteloric and has a wide nose.
D. (From C, the fetus on the right). Lack of occipitofrontal bossing in a case of trisomy 21.

PLATE III

PLATE IV

A. Normal thick placenta from a therapeutic abortus (8 weeks DA) showing abundant, well-vascularized chorionic villi and chorial plate.
B. Fragments of the same normal placenta.
C. Thin placenta from a 46,XX empty sac showing focal clubbing and reduced villi.
D. Another example of a thin placenta showing clubbing and cystic change of villi (9 weeks DA).

PLATE IV

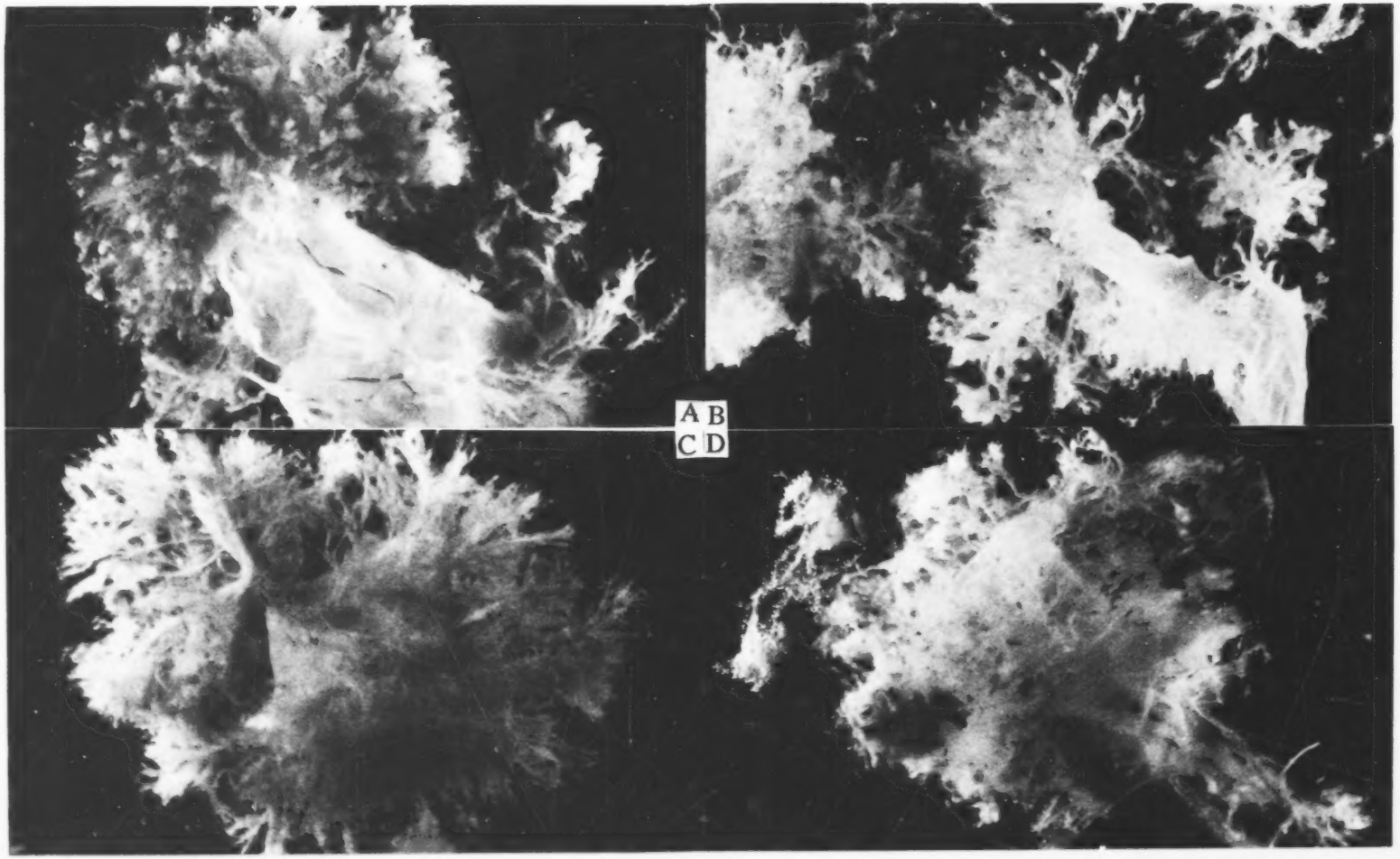


PLATE V

A. Markedly thin placenta exhibiting villous clubbing (9 weeks DA).
B. Thin trisomy 22 placenta with poorly branched, hypovascular villi.
C. PHM (69,XXX) showing a mixed pattern with some cystic change (arrow) of the villi.
D. CHM (46,XX) showing complete cystic formation of villi.

PLATE V

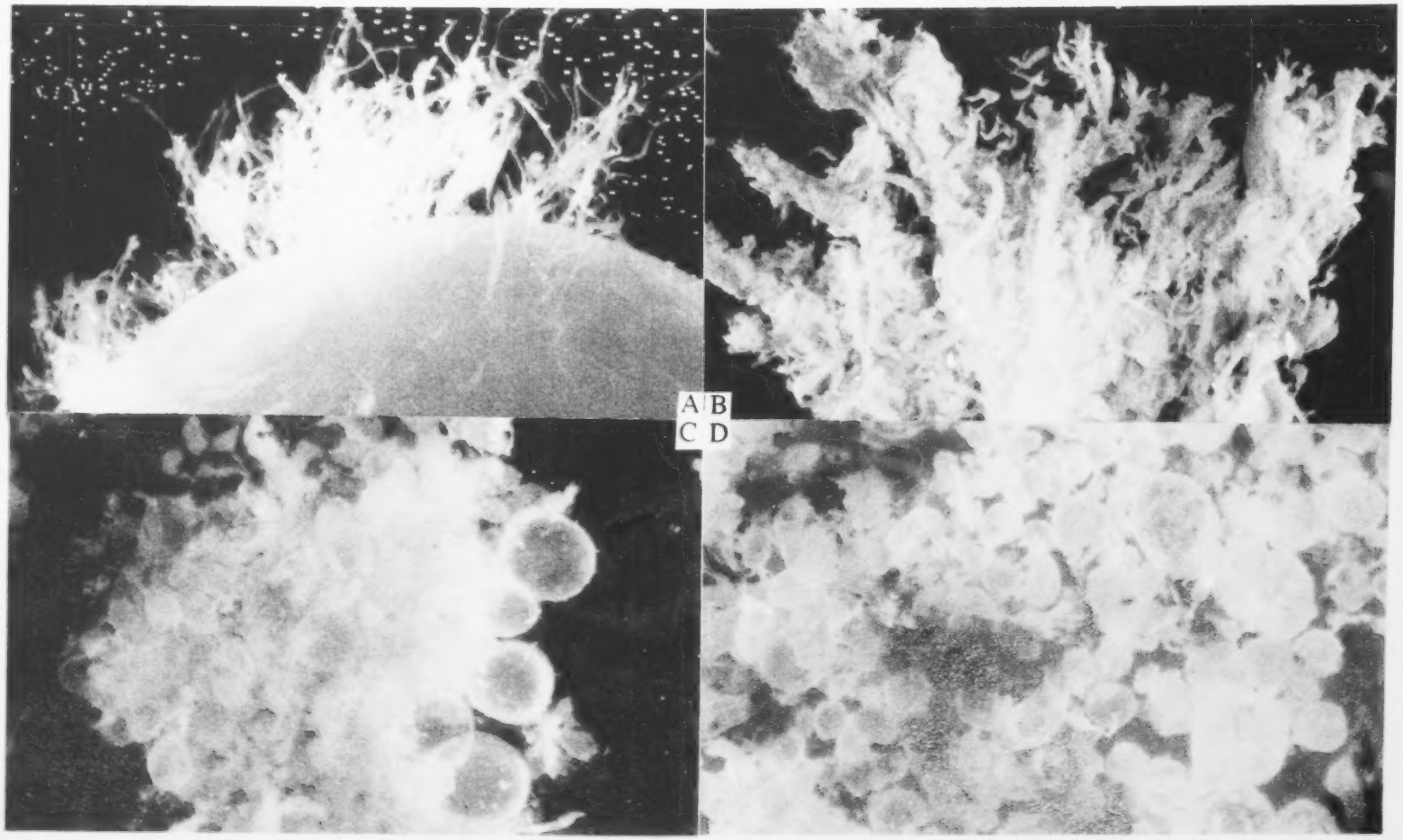


PLATE VI

A. Normal placenta at 2.5x. Abundant amnion (thin arrow) and chorion with well-formed chorionic vessels (wide arrow).

B. Normal well-vascularized chorionic villi at 16x.

C. In comparison to [A], chorial plate and amnion from a trisomy 22 conceptus at 16x, with poorly developed slit-like vessels in the chorial plate(arrows).

D. In comparison to [B], a hydropic villus with a thin trophoblastic lining seen here from a trisomy 22 conceptus.

PLATE VI

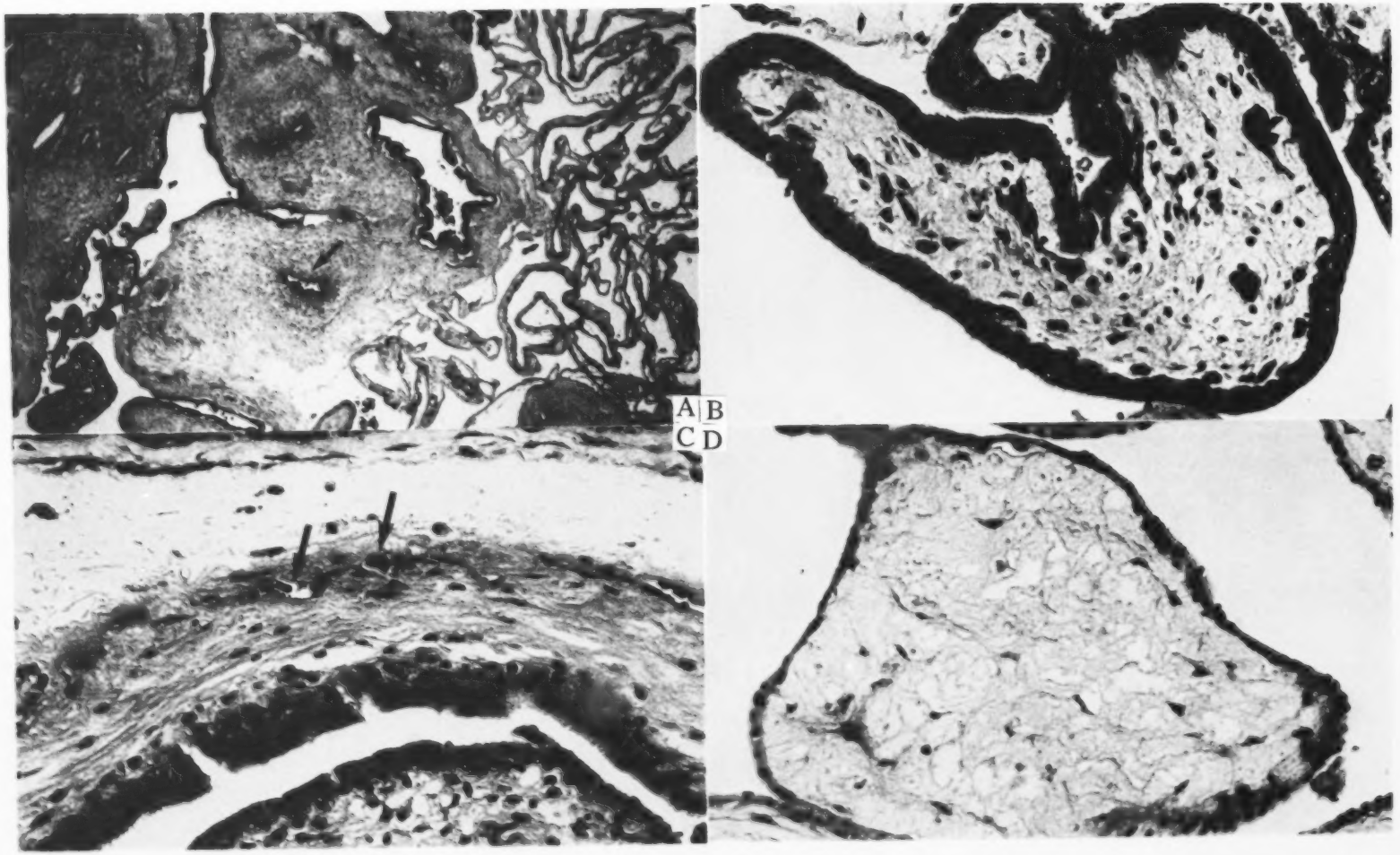


PLATE VII

A. Small compact villi seen in monosomy X (10x).
B. Intrastromal trophoblastic buds (arrow) in a trisomy 22 placenta (16x).
C. Intrastromal cytotrophoblastic cells (arrow) observed occasionally in trisomy (25x).

PLATE VII

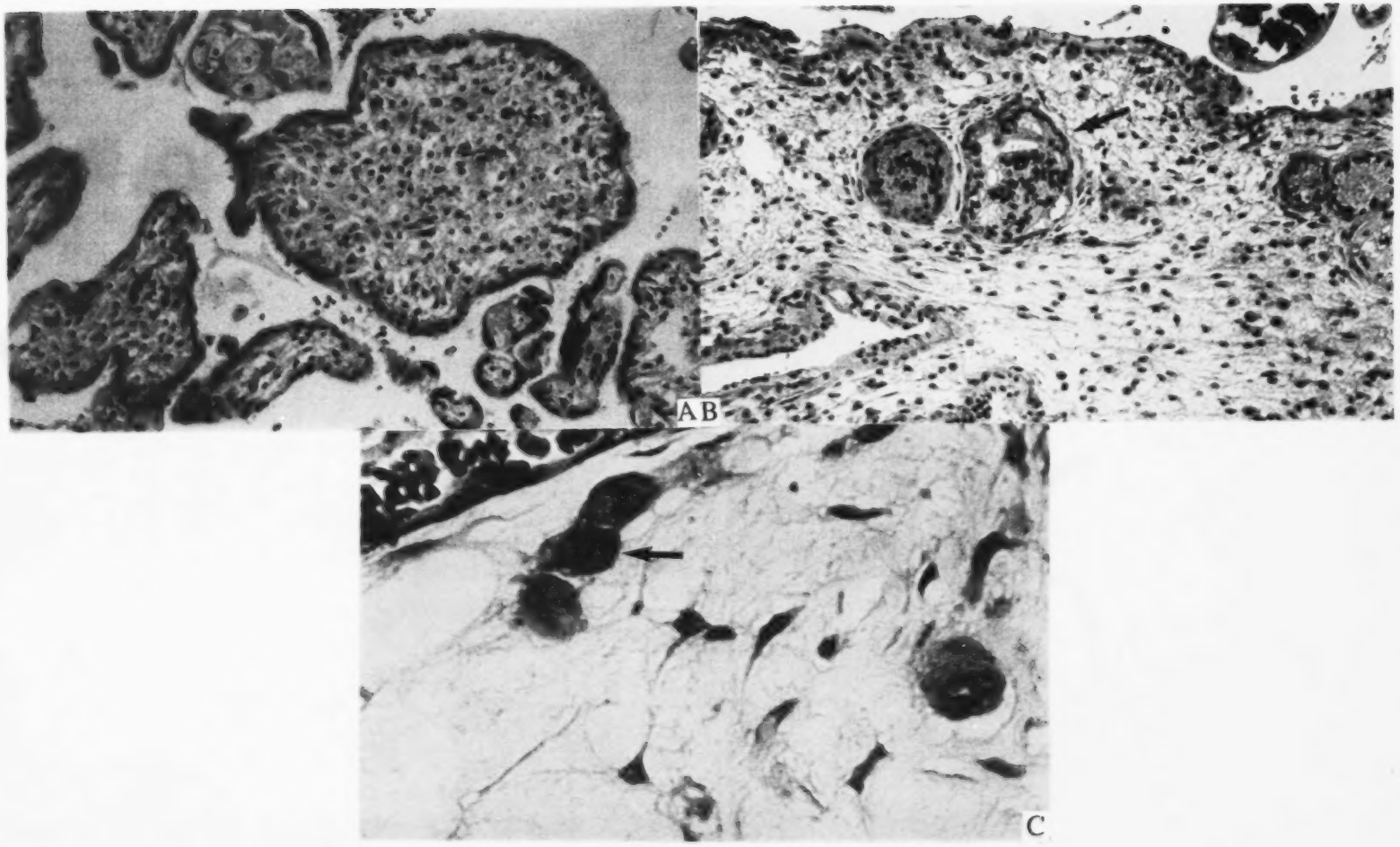


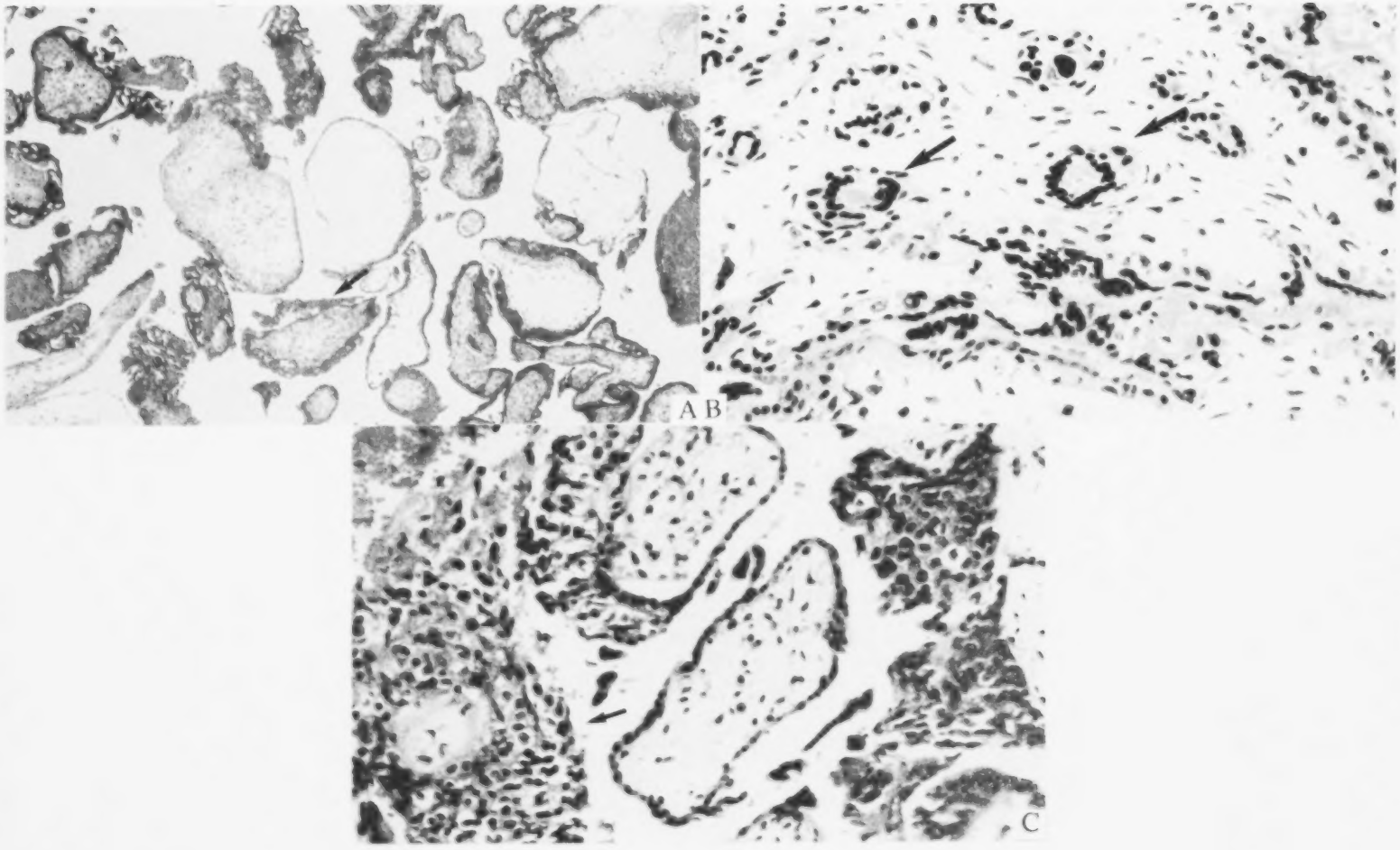
PLATE VIII

A. Histology of a PHM showing hydropic villi with some cystic change, and excess syncytiotrophoblast (arrow; 2.5x).

B. Hyperchromatic buds (arrow) in a non-molar triploid (10x).

C. Hyperchromatic stromal and trophoblastic nuclei in a tetraploid conceptus. Note the presence of numerous trophoblastic islands (arrow) and avascular villi.

PLATE VIII



CHAPTER 6

ANTHROPOMETRY IN THE PREVIABLE STAGES OF HUMAN DEVELOPMENT

6.1 Introduction

This series of data collection and analyses was designed to quantitate normal growth patterns in previable embryos and fetuses up to twenty weeks developmental age. Data were extracted from the pathology reports of archival specimens (458), and were obtained from direct measurements from prospective specimens (11). The results of this study were used as the basis for comparison with several heteroploid, cytogenetically-confirmed populations, i.e., trisomies 13, 18, 21, 45,X, and 3n. These studies were designed to reveal changes in growth by assessing growth coordination and rates of previously defined growth parameters (see section 6.2).

6.2 Experimental Protocol

The populations used in this study are as outlined in section 3.4, and are presented with their crown rump length and estimated developmental age in Table 6.1. As these specimens were collected over the last decade, not all parameters were measured for every specimen. Thus, the number of observations noted for parameters of a single population usually vary below the net population size for that group.

To conduct a comprehensive anthropometric analysis, it was necessary to establish a full set of external parameters measurable in control and study populations. The measurements were taken over the five body segments (craniofacies, neck, trunk, limbs, anogenital) and include linear and derived parameters (Table 6.2) which integrate metric traits to indicate growth in the second and third dimensions (Appendix B).

The data collection forms (Appendix A) were used in order to enter all data onto a computer spreadsheet (Microsoft Excel, version 3.0). Once entered, the spreadsheets were converted for use with the Statistical Package for Social Sciences (SPSS). This advanced package was used to compute Pearson correlation coefficients for all populations, perform the required regression analyses, Z-scores, and homogeneity tests using linear and some derived parameters. For the specific indices, mean values for each population were obtained and compared to normal using t-tests. The Z-scores were used to compare Pearson correlation coefficients of control and study populations, and the Cochran and Bartlett homogeneity test was used to examine the equality of the variances of the two populations.

Before interpreting the results of the experimental populations, it was necessary to demonstrate that

> (a) the state of the tissue, whether fresh or macerated, would not have a statistically significant effect on anthropometric data, and

Table 6.1: Anthropometric Populations and CRL

Population	CRL (mm)	EDA (days)
Normal, Fresh (n=302)	5-180	32-137
Normal, Macerated (n=47)	25-181	54-138
Normal, Karyotyped TA (n=27)	NA	NA
Trisomy 21 (n=41)	5-190	32-140
Trisomy 13 (n=7)	72-104	76-92
Trisomy 18 (n=9)	69-165	74-125
Monosomy X (n=21)	10-155	39-119
Triploidy (n=15)	12-119	30-100

EDA based on CRL

Table 6.2: Linear Anthropometric Parameters

Segments	Subsegments
Craniofacies	cranium: OFD, BPD, HC, CH; CFH face: FH, FW, OD, ICD, NL, NW, EL, EW, OL, Mouth
Neck	NeL, NeW
Upper limb Lower limb	UA, FA, H, F, Thumb T, LL, FL, Toes
Trunk	CC, AC, PC, IND
Anogenital	AGD, EgL, EgW

Additional, derived parameters are as described in Appendix B

(b) the normal population (n = 302) was not statistically different from the population of karyotypically and anatomically normal fragmented therapeutic abortuses. In order to do this, these populations were analyzed using SPSS, for the same previously discussed statistics. There was no statistically significant difference amongst the normal, fresh spontaneous and the karyotyped therapeutic abortus populations; some changes will be demonstrated with the macerated normal population.

No statistically significant differences exist between the normal fresh spontaneoous and fragmented therapeutic abortus populations, further supporting the categorization of the fresh fetuses as normal. Only minimal differences (Section 6.3A) exist between the normal, fresh and normal, macerated populations, enabling the combined analysis of fresh and macerated heteroploid embryos and fetuses of like genotype in a single anthropometric database.

6.3 Results

A. Normal Development

The results of Pearson correlation coefficients for the normal, (fresh) database (Table 6.3) revealed that normal development is highly regulated and strongly correlated amongst all linear parameters. Certain parameters showed stronger relationships than others, including crown rump length, crown heel length, and foot length. A very weak correlation, however, was seen with neck length (refer to Table 6.3); neck width was more tightly correlated.

Certain derived parameters which relate lean body mass to body weight, body mass index and ponderal index, showed tight correlations which were numerically consistent with the formulae from which they are derived (Appendix C). Body mass index is a ratio where the denominator is expressed to a second-order power. Thus, it is not surprising that this parameter correlates less tightly than other linear parameters. Ponderal index, on the other hand, is also a ratio where there is a cubic exponent in the denominator. Fully consistent with the formula, the associated correlation coefficients are both low and negative (Appendix C).

In the normal fresh population, 46 were embryos, 149 were male fetuses and 107 were female fetuses. No statistical difference was found amongst the male and female fresh fetus populations other than an increased rate of growth of the anogenital distance in male fetuses (figure 6.1).

Comparison of "diploid" fresh and macerated embryos and fetuses demonstrated strong correlations amongst all parameters, except for correlations involving the cranium. Although the growth rates' (assessed from the slopes of the regression equations) homogeneity of variances were statistically insignificant, the z-scores, which detect differences between correlation coefficients, indicate that significant differences affect occipitofrontal diameter and biparietal diameter. However, given that macerated embryos

and fetuses may experience distortion during retention and expulsion, this finding is in line others, including crown rump length, crown heel length, and foot length. A very weak correlation, however, was seen with neck length (refer to Table 6.3); neck width was more tightly correlated.

Certain derived parameters which relate lean body mass to body weight, body mass index and ponderal index, showed tight correlations which were numerically consistent with the formulae from which they are derived (Appendix C). Body mass index is a ratio where the denominator is expressed to a second-order power. Thus, it is not surprising that this parameter correlates less tightly than other linear parameters. Ponderal index, on the other hand, is also a ratio where there is a cubic exponent in the denominator. Fully consistent with the formula, the associated correlation coefficients are both low and negative (Appendix C).

In the normal fresh population, 46 were embryos, 149 were male fetuses and 107 were female fetuses. No statistical difference was found amongst the male and female fresh fetus populations other than an increased rate of growth of the anogenital distance in male fetuses (figure 6.1).

Comparison of "diploid" fresh and macerated embryos and fetuses demonstrated strong correlations amongst all parameters, except for correlations involving the cranium. Although the growth rates' (assessed from the slopes of the regression equations) homogeneity of variances were statistically insignificant, the z-scores, which detect differences between correlation coefficients, indicate that significant differences affect occipitofrontal diameter and biparietal diameter. However, given that macerated embryos and fetuses may experience distortion during retention and expulsion, this finding is in line with the gross appearance of these conceptuses. Retention promotes the softening of tissues like the brain, with collapse and then during expulsion there is further compression and distortion of the cranium, skeletal components like the limbs and facial skeleton are in no way affected by such changes. Consequently, although the z-scores are significant for occipitofrontal diameter and biparietal diameter, this is artifactual and not due to growth changes.

In contrast, comparison of the karyotyped therapeutic abortuses (27; fragments) database with that of the intact normal fresh revealed no significant findings for any of the parameters analyzed, further supporting our categorization of the normal, fresh conceptuses as anatomically and, most likely, karyotypically normal. Furthermore, the verification that anthropometric data are not altered by tissue status (fresh versus macerated), except for potential cranial distortion which can be deleted from data collection, permits one to group fetuses of like heteroploidy for analysis; this grouping is especially necessary in heteroploid populations where the samples are frequently small.

B. Trisomy 21

In comparison to the normal fresh population, multiple body segments showed statistically significant changes in trisomy 21.

Table 6.3: Pearson Correlation Coefficients For Normal Data (N = 302)

LINEAR PARAMETERS: INTERSEGMENTAL CORRELATIONS
GENERAL

	WEIGHT	CFH	TR	CRL	CHL
WEIGHT					
CFH	0.8042				
TR	0.9033	0.9012			
CRL	0.9016	0.8772	0.9683		
CHL	0.8930	0.8561	0.9535	0.9836	

GLOBAL

Craniofacial

	WEIGHT	CFH	TR	CRL	CHL
OFD	0.9038	0.8987	0.9505	0.9603	0.9547
BPD	0.8519	0.8598	0.8838	0.9219	0.9112
HC	0.8093	0.7807	0.8445	0.8714	0.8762
CH	0.6852	0.8680	0.8045	0.7144	0.6876
FH	0.8697	0.8895	0.8993	0.9366	0.9105
FW	0.7758	0.8063	0.8082	0.8684	0.8373
OD	0.8218	0.7419	0.8681	0.8730	0.8496
ICD	0.7759	0.7536	0.8163	0.8503	0.8437
NOSEL	0.8591	0.7832	0.8728	0.8965	0.8861
NOSEW	0.6750	0.5152	0.6480	0.6949	0.6975
EL	0.8613	0.7895	0.8648	0.9087	0.9040
EW	0.8053	0.6418	0.7224	0.8088	0.8081
UL	0.7614	0.7766	0.7547	0.8054	0.7802
MOUTH	0.7948	0.8017	0.7906	0.8174	0.8029

Neck

	WEIGHT	CFH	TR	CRL	CHL
NECKL	0.3179	0.4142	0.3767	0.4389	0.3754
NECKW	0.7281	0.7178	0.7554	0.7533	0.7431

TR

	WEIGHT	CFH	TR	CRL	CHL
CC	0.8803	0.7688	0.9108	0.9265	0.9376
AC	0.8951	0.7606	0.9274	0.9267	0.9322
PC	0.8352	0.7527	0.8466	0.8865	0.8899
IND	0.7037	0.6552	0.7340	0.6856	0.7073
SU	0.7002	0.7258	0.6777	0.7603	0.7091
IU	0.8713	0.8116	0.8413	0.8749	0.8580

Upper Limb

	WEIGHT	CFH	TR	CRL	CHL
UA	0.8960	0.9008	0.9565	0.9732	0.9686
FA	0.8936	0.8908	0.9605	0.9699	0.9627
HAND	0.8509	0.8400	0.8931	0.9252	0.9190
MIDFING	0.8133	0.6959	0.8304	0.8202	0.8036
LASTFING	0.8063	0.7066	0.8671	0.8224	0.8122

Lower Limb

	WEIGHT	CFH	TR	CRL	CHL
THIGH	0.8786	0.8512	0.9506	0.9301	0.9322
LLEG	0.8731	0.8631	0.9519	0.9438	0.9459
FOOT	0.8963	0.8717	0.8982	0.9381	0.9307

LINEAR PARAMETERS: INTERSEGMENTAL CORRELATIONS
SUBSEGMENTAL

Craniofacial

	OFD	BPD	HC	CH	FH	FW	OD	ICD	NL	NW	EL	EW	UL	MO
NeL	0.3606	0.3660	0.3290	0.2959	0.4610	0.4196	0.2471	0.2496	0.3871	0.1940	0.3552	0.2834	0.4986	0.3374
NW	0.7498	0.7138	0.6462	0.5896	0.7450	0.6588	0.7093	0.6568	0.7523	0.5893	0.7424	0.6577	0.6758	0.5582
CC	0.9165	0.8872	0.8520	0.6561	0.8437	0.7564	0.7960	0.8030	0.8520	0.6751	0.8802	0.7808	0.7614	0.7194
AC	0.9049	0.8622	0.8201	0.6551	0.8496	0.7558	0.7826	0.7920	0.8428	0.6522	0.8810	0.7831	0.7867	0.7398
PC	0.9185	0.8631	0.8593	0.6730	0.7953	0.6897	0.7038	0.7111	0.8066	0.6329	0.8350	0.7129	0.7230	0.7800
IND	0.6768	0.5966	0.3493	0.4933	0.6708	0.6741	0.5235	0.4256	0.6418	0.3617	0.6213	0.5166	0.6255	0.4399
SU	0.7334	0.6847	0.5882	0.5755	0.7436	0.6699	0.6680	0.5272	0.6089	0.4998	0.6416	0.5831	0.6002	0.6053
IU	0.9039	0.8699	0.8426	0.7072	0.8617	0.8345	0.7517	0.7610	0.8520	0.5208	0.7289	0.6777	0.7969	0.7241
UA	0.9562	0.9135	0.8605	0.7932	0.9074	0.8596	0.8586	0.8395	0.9127	0.7060	0.9067	0.8182	0.7893	0.8050
FA	0.9524	0.9081	0.8640	0.7856	0.9081	0.8575	0.8601	0.8406	0.9176	0.7167	0.9145	0.8260	0.7812	0.8002
HA	0.8922	0.8577	0.8135	0.7436	0.8917	0.8472	0.8410	0.8216	0.8525	0.7167	0.8830	0.8043	0.7564	0.7607
MF	0.7657	0.7144	0.6465	0.6714	0.7364	0.6353	0.7541	0.6698	0.7700	0.6554	0.7547	0.7090	0.6547	0.6948
LF	0.7739	0.7183	0.6682	0.6312	0.7386	0.6333	0.7372	0.6484	0.7602	0.6449	0.7461	0.6929	0.6423	0.7021
TH	0.8885	0.8383	0.7925	0.7395	0.8562	0.8012	0.8233	0.8058	0.903	0.6933	0.8904	0.8040	0.7969	0.7943
LL	0.9022	0.8538	0.8060	0.7408	0.8684	0.7939	0.8004	0.8075	0.9008	0.6803	0.8893	0.7959	0.7991	0.7974
FO	0.9173	0.8705	0.8066	0.7549	0.9038	0.8462	0.8697	0.8177	0.8773	0.7162	0.8951	0.8100	0.7546	0.8013

Neck

	NeL	NeW
CC	0.6693	0.8520
AC	0.2460	0.6697
PC	0.3187	0.6639
IND	0.1388	0.4299
SU	0.6300	0.5735
IU	0.5157	0.6370
UA	0.4420	0.7546
FA	0.4280	0.7427
HA	0.3950	0.7083
MF	0.2520	0.6485
LF	0.2901	0.6589
TH	0.3776	0.7415
LL	0.3505	0.7406
FL	0.4293	0.7126

TR

	CC	AC	PC	IND	SU	IU
UA	0.9032	0.9027	0.8619	0.7066	0.7082	0.8734
FA	0.9066	0.9109	0.8462	0.7015	0.7098	0.8759
HA	0.8585	0.8579	0.8352	0.6111	0.7006	0.8028
MF	0.7064	0.7020	0.6673	0.5378	0.5852	0.7181
LF	0.7021	0.7086	0.6745	0.5307	0.5591	0.7172
TH	0.8954	0.8988	0.8248	0.6053	0.6336	0.8500
LL	0.8867	0.8871	0.8336	0.6187	0.6423	0.8540
FL	0.8817	0.8751	0.8800	0.5459	0.7213	0.7921

Upper Limb

	UA	FA	HA	MF	LF
TH	0.9397	0.9376	0.8777	0.8367	0.8417
LL	0.9558	0.9483	0.8919	0.8477	0.8562
FL	0.9286	0.9254	0.9121	0.7849	0.7722

Lower Limb: refer to preceding tables of subsegmental development.

LINEAR PARAMETERS: INTRASEGMENTAL CORRELATIONS

Craniofacial

	OFD	BPD	HC	CH	FH	FW	OD	ICD	NL	NW	EL	EW	UL	Mo
OFD														
BPD	0.9375													
HC	0.8911	0.8652												
CH	0.7412	0.6824	0.6146											
FH	0.9194	0.8823	0.8384	0.7293										
FW	0.8163	0.8639	0.7520	0.6672	0.874									
OD	0.8292	0.7811	0.7509	0.5923	0.8212	0.7972								
ICD	0.8322	0.7821	0.7406	0.6345	0.8145	0.7583	0.8137							
NL	0.8616	0.8462	0.8224	0.6109	0.8577	0.8071	0.7921	0.8091						
NW	0.6451	0.6303	0.6022	0.3803	0.6402	0.5807	0.7023	0.6334	0.7026					
EL	0.8902	0.8445	0.8296	0.6284	0.8765	0.8216	0.8221	0.8008	0.8732	0.7009				
EW	0.7423	0.7018	0.7352	0.4916	0.7470	0.7063	0.7921	0.6782	0.7659	0.7405	0.8303			
UL	0.7528	0.7270	0.7139	0.6485	0.7755	0.6681	0.7375	0.6313	0.7498	0.5731	0.7609	0.6090		
Mo	0.8066	0.7548	0.7335	0.7000	0.7227	0.6036	0.7248	0.6803	0.7273	0.5254	0.7578	0.6216	0.6437	

Neck*

	NL
NW	0.3187

TR

	CC	AC	PC	IND	SU	IU
CC						
AC	0.9373					
PC	0.9123	0.9186				
IND	0.6300	0.7454	0.5085			
SU	0.6778	0.6574	0.6688	0.3666		
IU	0.8237	0.7751	0.8154	0.7237	0.7500	

Upper Limb

	UA	FA	HA	MF	LF
UA					
FA	0.9891				
HA	0.9258	0.9142			
MF	0.8222	0.8240	0.7867		
LF	0.8180	0.8204	0.7612	0.9515	

Lower Limb

	TH	LL	FOOT
TH			
LL	0.9940		
FOOT	0.9072	0.9049	

DERIVED PARAMETERS: INTERSEGMENTAL CORRELATIONS

GLOBAL

	WEIGHT	CFH	TR	CRL	CHL
BMI	0.2294	0.7342	0.8168	0.7882	0.0688
PI	- 0.0912	- 0.2686	- 0.0470	- 0.3338	- 0.2127
MIDFAC A	0.8660	0.8241	0.8342	0.8676	0.8525
CRAN S	0.9307	0.8760	0.9183	0.9229	0.9194
CRAN V	0.8945	0.8952	0.8507	0.7981	0.7967
TR S	0.9437	0.8091	0.9639	0.9263	0.9251
TR V	0.9647	0.7706	0.9279	0.8943	0.9029

DERIVED PARAMETERS: INTERPARAMETRIC CORRELATIONS

Craniofacial

	OFD	BPD	HC	CH	FH	FW	OD	ICD	NL	NW	EL	EW	UL	MO
BMI	0.7930	0.7863	0.7357	0.6537	0.7743	0.7575	0.7558	0.6924	0.8144	0.6098	0.7602	0.6955	0.6883	0.7114
PI	-.2778	-.2297	-.0921	-.1617	-.2580	-.1460	-.0745	-.2554	0.0296	-.0423	-.2109	-.1623	-.0218	0.0024
MF	0.8569	0.8613	0.7803	0.6907	0.9382	0.9500	0.8023	0.7528	0.8218	0.6071	0.8511	0.7422	0.7238	0.6536
CSA	0.9619	0.9592	0.8734	0.7073	0.8976	0.8224	0.8088	0.7744	0.8511	0.6375	0.8740	0.7407	0.7475	0.7911
CV	0.8531	0.8180	0.7736	0.8816	0.8074	0.7099	0.7185	0.6683	0.7448	0.5226	0.7678	0.6466	0.7149	0.7681
TSA	0.9417	0.8817	0.8267	0.9367	0.8463	0.6425	0.8253	0.7048	.8195	0.6183	.8569	0.7083	0.7992	0.7389
TV	0.9204	0.8348	0.7876	0.9208	0.8134	0.5962	0.7817	0.6394	0.7517	0.5691	0.8187	0.6655	0.7974	0.7301

Neck

	NeL	NeW
BMI	0.3260	0.69344
PI	0.0338	- 0.0053
MIDFAC A	0.4312	0.6957
CRAN S	0.3532	0.7325
CRAN V	0.2926	0.6939
TR S	0.1920	0.6795
TR V	0.1770	0.6483

TR

	CC	AC	PC	IND	SU	IU
BMI	0.7981	0.8338	0.7741	0.5728	0.6260	0.7328
PI	- 0.1214	- 0.0853	- 0.1146	0.0634	0.0418	- 0.1063
MIDFAC A	0.8084	0.8100	0.7399	0.7003	0.7374	0.8843
CRAN S	0.9113	0.8904	0.8955	0.6655	0.7228	0.9071
CRAN V	0.8262	0.8038	0.8190	0.6248	0.6958	0.8834
TR S	0.9693	0.9340	0.8588	0.8312	0.6997	0.8456
TR V	0.9023	0.9418	0.8511	0.8592	0.6779	0.7629

Upper Limb

	UA	FA	HA	MF	LF
BMI	0.1434	0.1505	0.1259	0.6804	0.6654
PI	- 0.1473	- 0.1395	- 0.1513	- 0.0517	- 0.0625
MIDFAC A	0.8554	0.8599	0.8610	0.6952	0.6918
CRAN S	0.9232	0.9177	0.8677	0.7624	0.7690
CRAN V	0.8370	0.8268	0.8047	0.7642	0.7452
TR S	0.9191	0.9198	0.8190	0.7637	0.8038
TR V	0.8822	0.8690	0.8027	0.7379	0.7887

Lower Limb

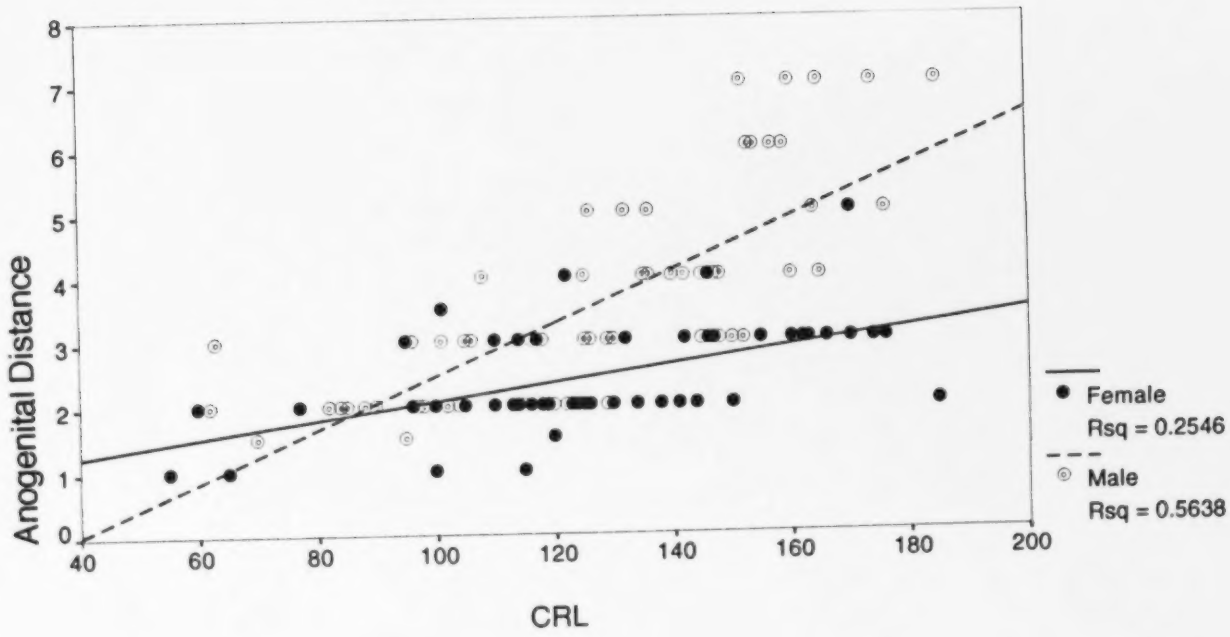
	TH	LL	FOOT
BMI	0.7937	0.7874	0.8035
PI	- 0.3086	- 0.3048	- 0.2303
MIDFAC A	0.8178	0.8058	0.8803
CRAN S	0.8603	0.8722	0.8977
CRAN V	0.7897	0.7985	0.8254
TR S	0.8881	0.8846	0.8597
TR V	0.8620	0.8567	0.8000

DERIVED PARAMETERS: INTRAPARAMETRIC CORRELATIONS

GENERAL

	BMI	PI	MFAC A	CRAN S	CRAN V	TR S	TR V
BMI							
PI	0.9347						
MFAC A	0.7892	-.1111					
CRAN S	0.8136	-.1674	0.8788				
CRAN V	0.7829	-.0920	0.8116	0.9029			
TR S	0.7810	0.0518	0.7674	0.9367	0.8851		
TR V	0.7745	0.0540	0.7333	0.9208	0.8851	0.9661	

Figure 6.1: Normal Anogenital Distance
in Fresh Males and Females



1) reduction in the growth rate of the calvarial volume in spite of non-significant changes in biparietal diameter, occipitofrontal diameter and calvarial height, the metric traits used to derive this parameter

2) reduction in nasal index (length/width), related to a statistically significant increase in nasal width

3) reduced growth rate of ear length, without affecting the aural index (length/width), (figure 6.2)

4) increased variability of IPD/BPD and IPD/FW, which are indirect teloric indices, in contrast to the intercanthal diameter which is a direct index.

5) decrease in the facial index (= facial height/facial width), despite non-significant changes in the parameters which determine this index.

The phenotypic variability in Down's syndrome, which has been quantitated by anthropometry has been of interest as a model for the investigation of the effects of aneuploidy on developmental pathways (Shapiro, 1970). Typically, the systems thought to be most affected are those which show greater variability in normal development (Barden, 1980). Examples of such traits include dental morphology (Shapiro, 1970), palate (Shapiro, *et al.*, 1967; Shapiro, 1975) and dermatoglyphics (Shapiro, 1975).

In order to analyze anthropometric variability, which would have resulted if there had been amplified developmental instability in this population, the Levene's test of variance within a single population was conducted on the trisomy 21, 45,X and 3n populations and for comparison, the normal, fresh, population. The results for trisomy 21 are shown in Table 6.4. The statistic rank orders all measured parameters by variability within a single population to yield a continuum of increasing parametric variability as one approaches the bottom of Table 6.4. If Shapiro's theory was correct, a mostly similar ranking would be expected for both populations since only the most variable parameters in the normal population would show greater variability in the trisomy 21 population. This, however, was not found. In fact, the parameters showed no consistent order amongst the two populations.

The homogeneity of variance test, employed to analyze the variance of individual parameters, demonstrated increased variability for:

1) calvarial volume
2) cranial index
3) cervical index
4) aural index
5) cervical length

and does not show any direct relationship to the ranking of the Levene's test.

Trisomy 21 fetuses show short upper limbs as indicated by slope changes in:

1) a reduced growth rate of the complete upper arm (figure 6.3)
2) reduced growth rate of the forearm (figure 6.4)

Figure 6.2: Ear Length
in Trisomy 21 vs. Normal

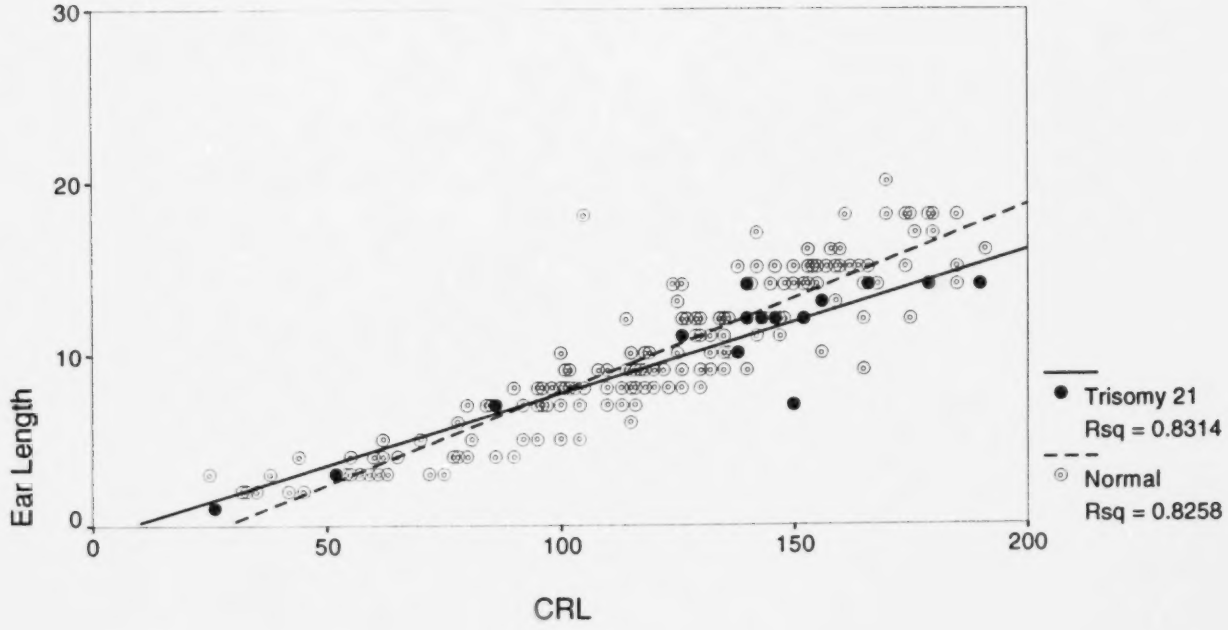


Figure 6.3: Upper Arm Length
in Trisomy 21 vs. Normal

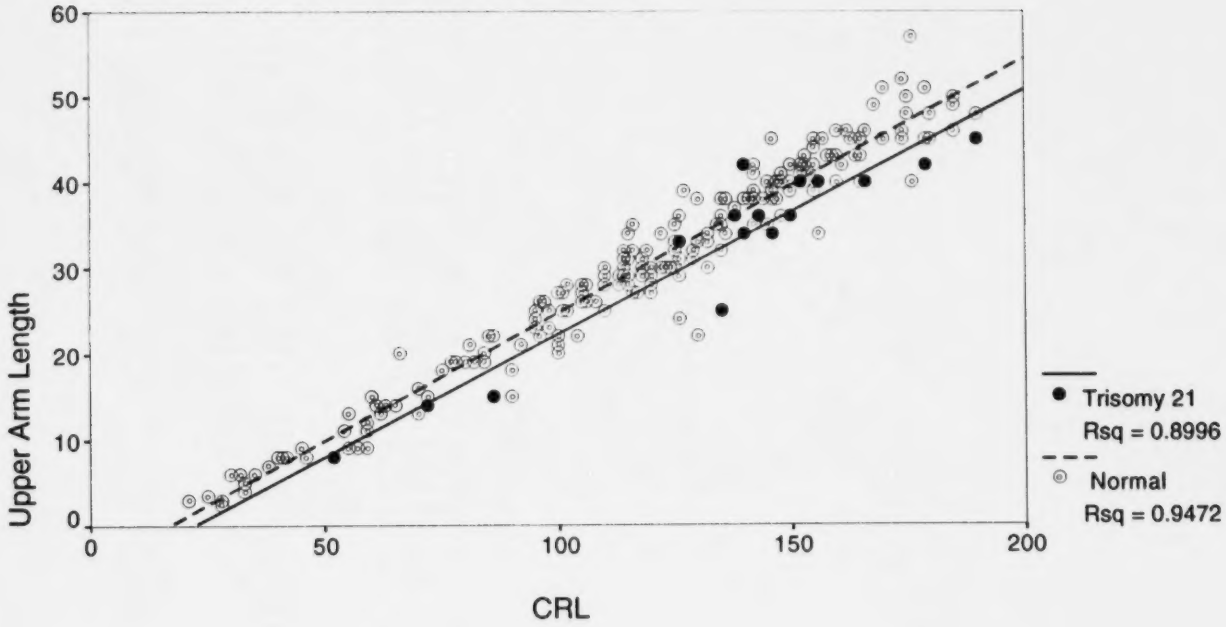


Figure 6.4: Forearm Length
in Trisomy 21 vs. Normal

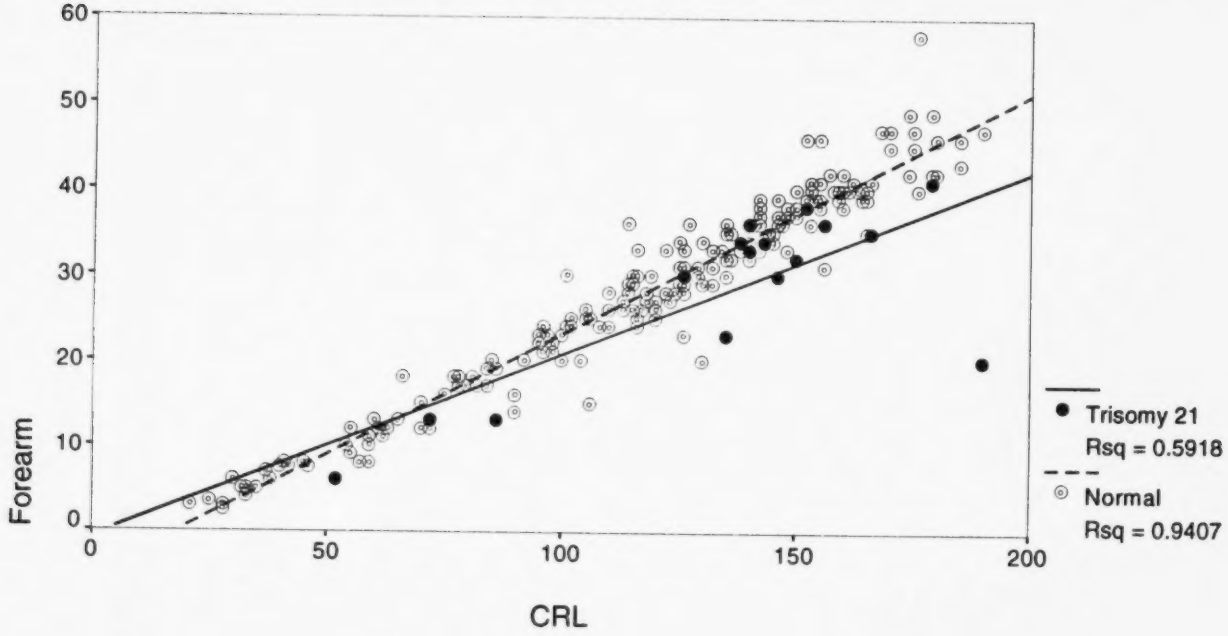


Table 6.4: LEVENE'S TEST FOR THE MEASUREMENT OF VARIANCE AMONGST PARAMETERS OF A SINGLE POPULATION

NORMAL	TRISOMY 21
Fac Area	Fac Area
IntLim	Mouth
Foot	BPD/OFD
Thigh	NasInd
TLLL	ICD
AGD	BMI
LLeg	NeckInd
BPD/OFD	FacInd
Hand	EarInd
CC	UCDiam
NasInd	UArm
IND	DAbuvPS
PC	CC
MidFinger	NoseW
NoseW	Orbit
EgL	CHL
CHL	EarL
CranVInd	FA
NeckInd	EarW
LastFing	AbC
Litoe	CFH
Mouth	CRL
UArm	DBeloXS
AbC	PI
Orbit	CranSA
EarL	BPD
DBeloXS	CranHt
FW	FH
EgW	TULL
OFD	EgW
BMI	CranVInd
CRL	NeckW
PI	HeadC
TULL	IntLim
TelInd	Hand
CranHt	Midfinger
NoseL	EgL
CFH	Litoe
NeckL	NoseL
FA	FW
NeckW	LLeg
FacInd	TLLL
BPD	Thigh
EarInd	OFD
DAbuvPS	TelInd
EarW	NeckL
UCDiam	Foot
HeadC	AGD
ICD	PC
CranSA	LastFinger
FH	IND

The lower limbs are not affected in the same manner, but the growth rate of the feet is reduced. This has specific implications for the gestational dating of these conceptuses which will be discussed further in section 6.4

C Trisomies 13 and 18

Pearson correlation coefficients (growth coordination) and z-scores (comparing correlation coefficients) could not be obtained for either of these populations because of the small population sizes (seven in trisomy 13, nine in trisomy 18) would not permit the pair-wise analysis necessary to obtain correlation coefficients. Thus, only regression equations (growth rates) and homogeneity tests (variability) could be computed. There were no statistically significant differences noted for either of these populations in comparison to normal.

D. 45,X

The anthropometric analysis of 21 45,X embryos and fetuses demonstrated increased variance and growth rate changes across multiple body segments. Increased variance (significant homogeneity of variance) was seen in:

1) aural width
2) cervical width

Growth rate (slopes) changes were seen in:

1) decreased aural length (figure 6.5)
2) decreased aural width (figure 6.6)
3) decreased upper lip length
4) increased cervical width
5) increased chest circumference (figure 6.7)

E. Triploidy

The fifteen conceptuses (four molar and eleven non-molar) in this population only showed reduced growth rate of the upper limbs as compared with normal and with trisomy 21 (figure 6.8).

6.4 Interpretation of Results

Normal human development is easily recognized. The analysis of normal development, involving fresh and macerated, spontaneous and therapeutic abortuses shows a highly regulated process where growth of body segments and subsegments are strongly correlated (>0.8) with each other with the exception of neck length, which for unknown reasons is extremely variable. Global parameters like crown heel length, crown

Figure 6.5: Ear Length in
Monosomy X vs. Normal

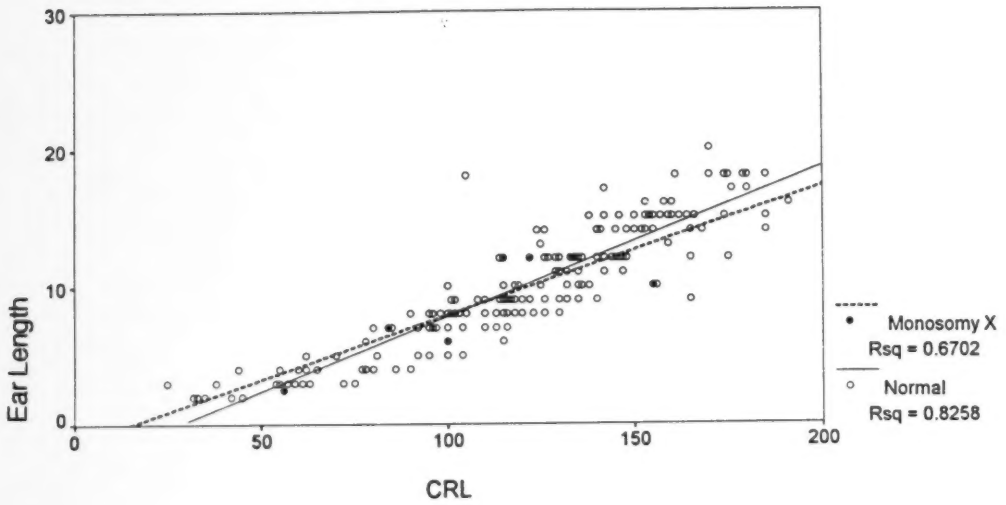


Figure 6.6: Ear Width in
Monosomy X vs. Normal

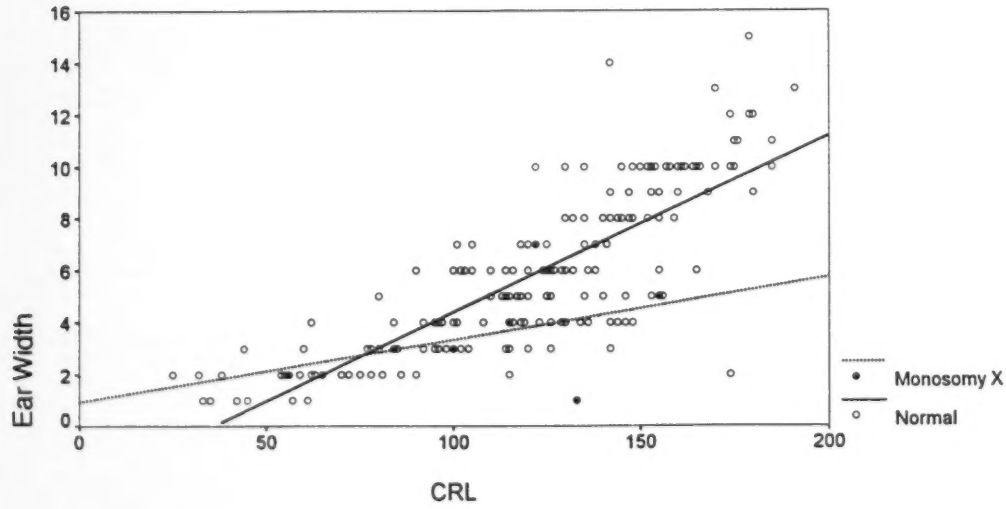


Figure 6.7: Chest Circumference
in Monosomy X vs. Normal

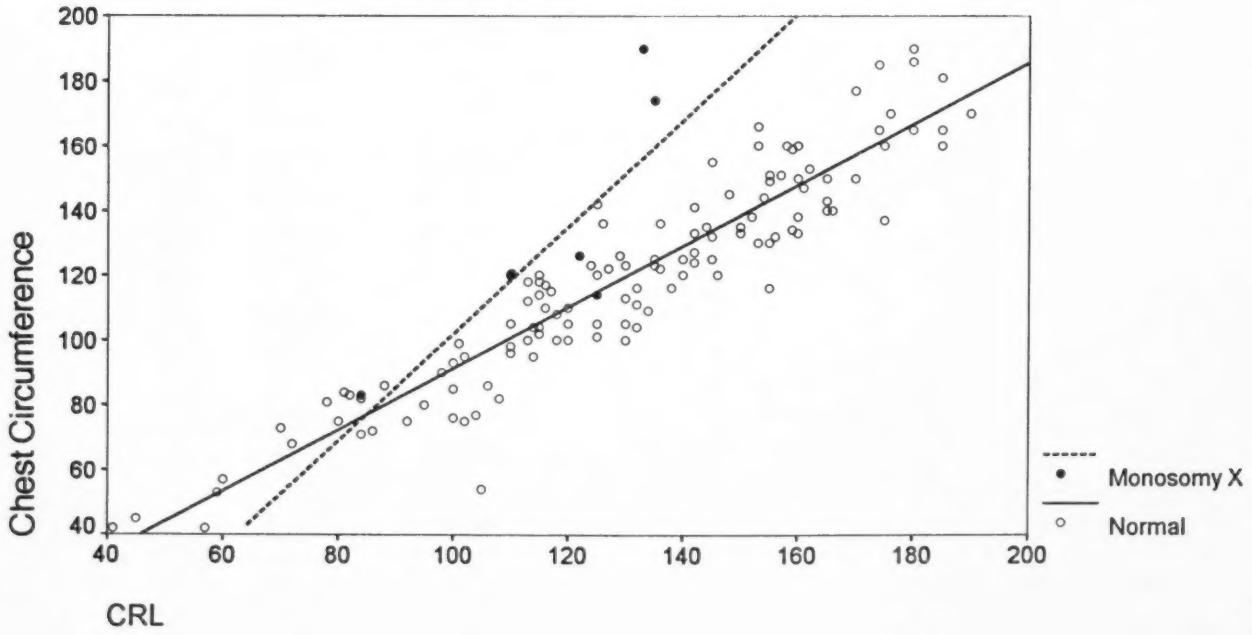
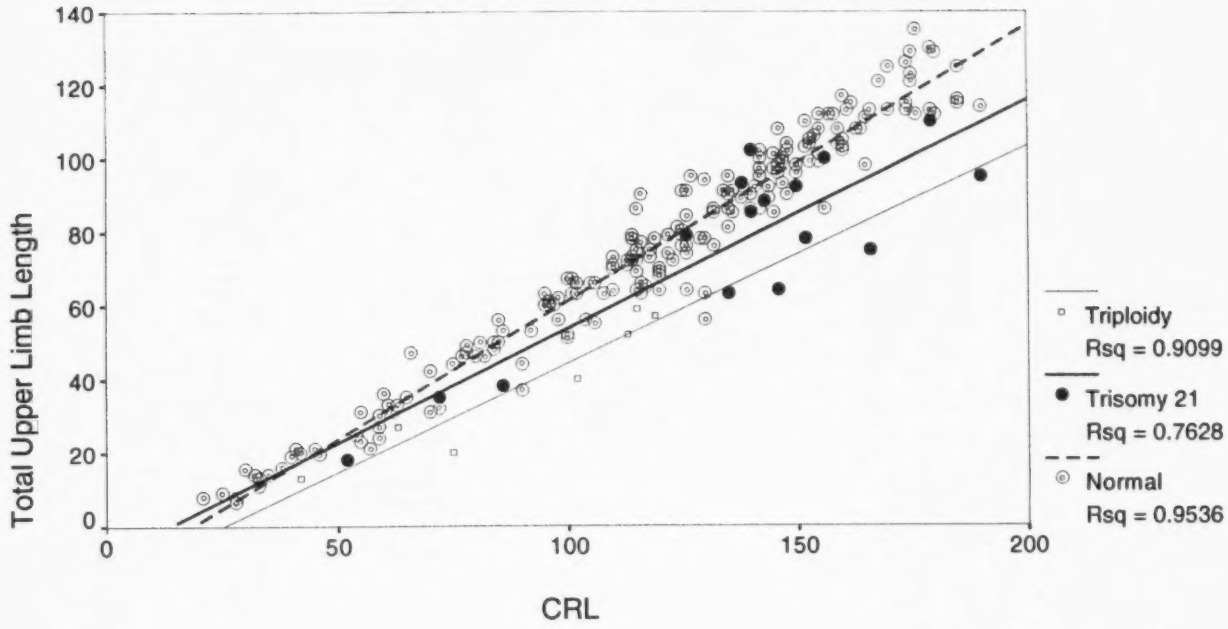


Figure 6.8: Total Upper Limb Length
in Triploidy vs. Normal



rump length, body weight and foot length show stronger correlations than others (0.9). In order to achieve strong correlations between global parameters, body segments and subsegments, regulation of growth rates must also be achieved. This was supported by the data which provided specific growth rates for parameters in relation to the recognized dating standard, crown rump length.

This assessment of normal development supports previous theories that have suggested that processes like canalization (Waddington, 1942) or developmental homeostasis (Lerner, 1954) exist wherein the expressions of genes and the environment have co-adapted to produce the "normal" phenotype. Furthermore, it is thought that the degree of canalization varies amongst developmental pathways (Shapiro, 1970), and permits greater variability in the size of specific traits than others in normal development. Although variability exists in the normal phenotype, the developmental process is highly regulated maintaining a recognizably normal conceptus.

Despite changes in tissue status, i.e. fresh or macerated, no changes in variances were observed. The only statistical difference amongst the population affected the cranium; this artifact was related to the softening of tissues leading to secondary calvarial collapse. Thus, where macerated and markedly distorted embryos and fetuses are involved in anthropometry, measurements should be omitted from severely distorted crania.

The analysis of trisomy 21 fetuses yielded multiple significant findings which involved subsegments of the craniofacial region and limbs. The positive findings closely parallel the phenotypic presentation of the trisomy 21 conceptus:

1) The reduction of the calvarial volume growth rate which was not accompanied by significant changes in biparietal diameter, occipitofrontal diameter, and calvarial height suggests that non-significant changes in these metric traits yield significant differences in the derived parameter. These changes are considered to be accurate in this population and not as a result of maceration because 6 intact fetuses in 20 were macerated and only one was distorted, making it unlikely that distortion was the cause of this finding. Also, the non-significant decrease in biparietal diameter may contribute to the lack of occipitofrontal bossing frequently observed in trisomy 21 (Plates IIIA, C, D).

2) The reduction in the nasal index and increase in nasal width correspond to the wide nose seen in these fetuses.

3) The reduction in ear length in trisomy 21, contributes to the rounded, as opposed to oblong appearance of the ear in monosomy X.

4) The increased variability of the indirect teloric indices IPD/FW, biparietal diameter but not of the direct teloric indices identify developmental instability in the intercanthal subsegment in trisomy 21. The direct teloric index is capable of detecting hypo- to hyper-telorism based on changes in the relationship between the orbit diameter and intercanthal distance. However, both indirect indices are influenced by changes in overall head shape which may give the illusion of normal to increased telorism based on changes in facial width and BPD. The increased variability of these parameters indicates that a unidirectional change to

hypertelorism exists in trisomy 21. This change is clearly visible in some cases but is not clearly identifiable in others. It is possible that indirect teloric indices do not demonstrate increased means in trisomy 21 as a result of the presence of fetuses which are near-normal; larger numbers may be required to identify a potential increase in the mean teloric (direct and indirect) indices.

5) The reduction in facial index (= height/width) corresponds to the overall rounded appearance of the face. The decreased index results despite non-significant changes in both facial height and facial width; this maybe the result of small numbers and small changes in early gestation.

The effect of a trisomy 21 genotype on the rate of foot growth is an important finding that should not be overlooked. The most popular anthropometric measurements for fetal dating are crown rump length and foot length. Given that crown rump length is not always available, as in cases of therapeutic abortion by suction curettage or dilatation and extraction where limb fragments are usually the only embryonic or fetal tissue obtained, this significant finding suggests that dating the trisomy 21 conceptus by foot length would not be a reliable method for determining gestational age leading to underestimation. This finding has the greatest impact on ultrasonography since fetal foot length in combination with other parameters is widely used for this purpose (Platt *et al.*, 1988).

The trisomy 13 and 18 study populations were too small, 7 and 9 fetuses respectively, to evaluate for growth coordination. Although regression and homogeneity of variance analyses provided no significant results, the characteristic phenotypes of these aneuploidies make it likely that a larger sample size would demonstrate distinct anthropometric findings. For example, short limbs is a phenotype associated with these populations but does not occur in all cases. Likewise, short limbs were not found in all or even the majority of these cases. However, a larger population would not only enable the pairwise analysis necessary to obtain Pearson correlation coefficients, it would also enable z-score (comparison of correlation coefficients) analysis, and improve regression and homogeneity of variance analyses. Small populations mask the ability to identify deviations from normal in the latter two tests.

The anthropometric analysis on 45,X revealed growth coordination changes that closely paralleled fetal phenotype. Increased growth rate of the cervical width and the increased variance were found which do not reflect growth changes in this population. Rather, these two significant findings are related to the incidence of cystic hygroma in 45,X (8 of 10 complete fetuses). The degree of severity of the cystic hygroma will not only affect the cervical width, but also the variation in this parameter. Furthermore, the increased growth rate would suggest a gradual increase over age. This is fully consistent with the pathogenesis of cystic hygroma, which results from fluid accumulation in the jugular lymph sacs. The fluid which accumulates is secondary to protein accumulation in the tissue (Levine, 1989), the cause of which is thought to be the failure of the jugular sacs to communicate with the venous system in the neck (Chervenack *et al.*, 1983). Consequently, this failure would lead to a progressive worsening of the cystic hygroma over time, lead to general hydrops (Smith, 1982), and would explain the lack of

identification of early cases of cystic hygroma. Aural length demonstrated an decreased growth rate as did aural width in 45,X. Differences in the actual growth rates might explain the disproportionate growth in opposing directions that would, then, account for the oblong, but not wide or round ears, that were seen in 45,X fetuses. This finding has not previously been reported, perhaps owing to the small size of the ear at this period of development and, possibly, lack of measurement. Nonetheless, in fetuses with cystic hygroma, the analysis of aural development would be a helpful discriminator, where round as compared to long ears would favour trisomy 21 over 45,X. The increased variance of the chest circumference in this population is considered to be secondary to the variable degree of subcutaneous edema which is frequently associated with 45,X development.

Phenotypically, triploid conceptuses are recognized as having micro- or hydrocephaly, short limbs and a thin, gracile body. This statistical analysis only detected a reduction in the growth rate of the upper limbs but no changes in other body segments. This series of conceptuses demonstrated predominantly microcephaly although hydrocephaly was also observed. It is likely that these changes had statistically opposing influences that diminished the ability to detect deviations related to the cranial region during analysis, a problem compounded by small numbers. Also, the lower limbs did not demonstrate statistically significant changes in growth rate because the changes that occurred were too small to detect. Furthermore, the circumferences required to analyze the gracile nature of the trunk were only available in 3 triploid conceptuses; they showed reduced circumferences as compared to normal. A larger sample size, with a full set of measurements, may provide enough information to better assess this population.

The assessment of variation of all metric traits analyzed with respect to Shapiro's theory was completed with the Levene's test of variability for a single population. If all metric traits of lower variability in normal development retained their relative positions as rank ordered by this test for the trisomy 21 population, while metric traits of greatest variability retained a similar but not exact position in the rank ordering, then the Levene's variability test would have demonstrated that the data support Shapiro's theory. However, table 6.4 shows that as variability increases (going down the table), the traits retain no fixed order with respect to the normal population; they appear in an entirely different sequence for trisomy 21, thus, not supporting Shapiro's contention. This study also attempted to extend Shapiro's theory to other forms of heteroploidy, however, insufficient data interfered with the assessment of these populations.

Shapiro employed Waddington's (1942) theory of canalization to explain the effect of heteroploidy on development, suggesting that it alters the stability of developmental pathways by reducing the degree of genetic control exerted on the pathway, leading to increased variability in those traits. Shapiro (1970) believed that this would account for increased variability of the most variable traits in normal development. However, contrary to Shapiro (1970), in each of these forms of heteroploidy some pathways like those which control facial height (see table 6.4), the most variable trait in normal development which was analyzed in this study, are more rigidly controlled in trisomy 21 resulting in the ranking of this metric trait near the middle of the range for this

form of trisomy; other metric traits like internipple distance (most variable in trisomy 21, but not in normal) show more variability as compared to normal.

CHAPTER 7

ALTERED PLACENTAL MORPHOMETRY REVEALED BY TWO-DIMENSIONAL IMAGE ANALYSIS

7.1 Introduction

Heteroploidy is recognized as affecting the entire conceptus, embryo and placenta (Boué *et al.*, 1976). Placental changes can be subtle or severe, but the more severe the placental phenotype is strongly correlated with poor embryonic development (Honoré *et al.*, 1976). These are very few morphometric studies of placentas from heteroploid conceptuses and they are in general agreement with the qualitative and semi-quantitative observations (Philippe, 1973; Röckelein *et al.*, 1989). The quantitation of such structural alterations in placental development was conducted at two levels (section 3.4). Quantitation of human placental development has been facilitated by the dissecting microscope and the availability of computer software for morphometric analyses. The purpose of this study was to quantitate placental changes resulting from heteroploidy at the gross and histologic levels using the dissecting microscope and a semi-automated computer software program, respectively.

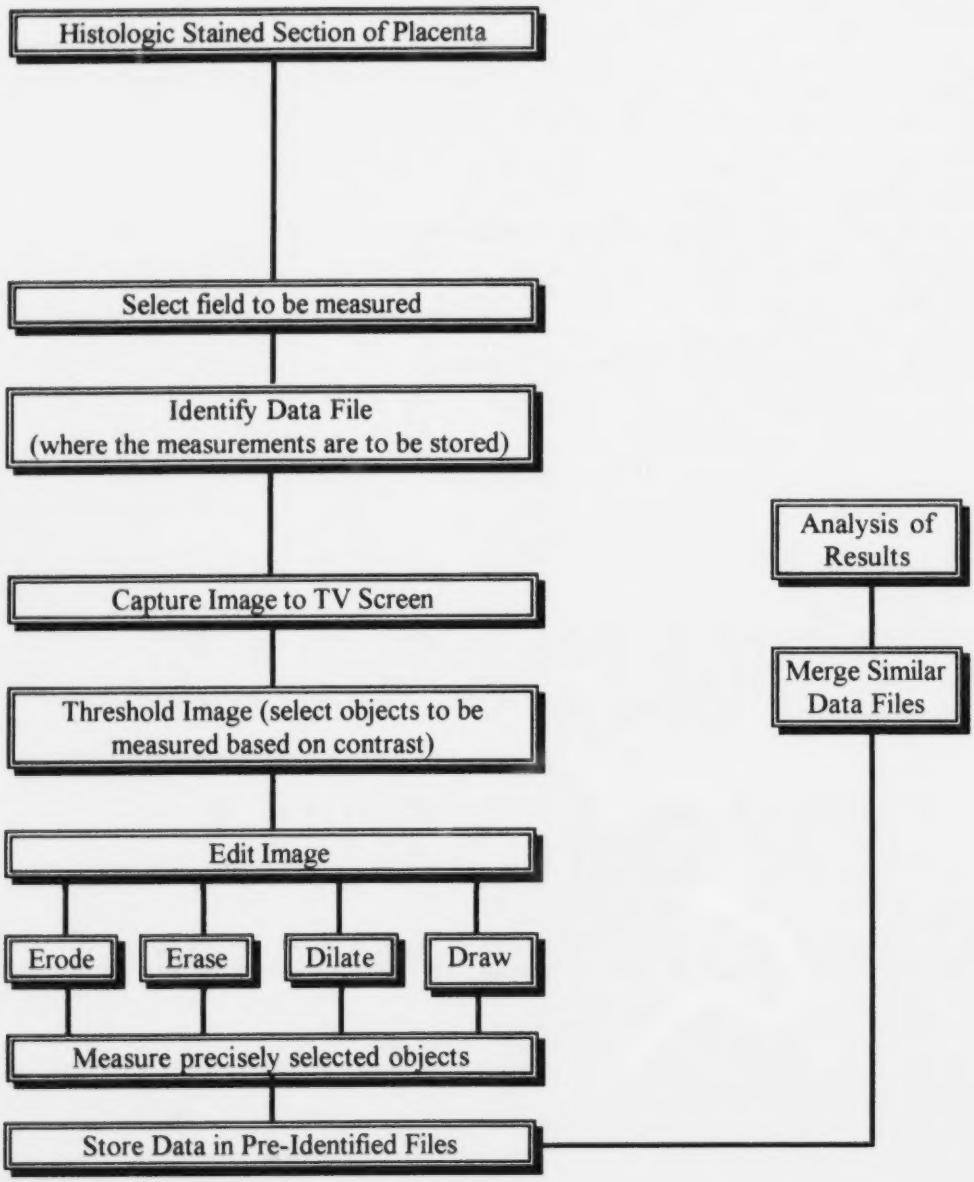
7.2 Experimental Protocol

Both techniques of morphometric analysis involved the novel utilization of an already existing technique. The analysis conducted with the dissecting microscope utilized a protocol which was employed only once previously (Bøe, 1967) for a qualitative gross analysis of single truncal systems from normal therapeutic abortuses; the purpose of using single truncal system analysis in the present study was to analyze villous development and differentiation as influenced by genotype. The study of populations used in this component were comprised of a total of 22 specimens, 5 of which were karyotyped, 12 were normal therapeutic abortuses and 5 were early, missed abortions without obvious abnormality. The fresh conceptuses ranged from 8-13 weeks DA. For each conceptus, available placental tissue was put in a petri dish with saline and examined under the dissecting microscope. Fine dissecting scissors and fine-tipped forceps were used to grasp and remove a single truncal system from the chorial plate. The single truncal system was then photographed and analyzed according to section 3.3C.

Placental quantitative analysis conducted with a semi-automated interactive computer program (Genias 25 by Joyce Loebl) has previously been conducted on term placentae (Franco *et al.*, 1987; Bouw *et al.*, 1978). However, it has never been utilized in a comparative fashion on normal and trisomic previable conceptuses (section 3.3D).

Through a series of sequential steps (Figure 7.1), the user will come to a menu which requests that parameters for measurement be identified. These are standard

Figure 7.1: The Mechanics of Semi-Automated Interactive Histomorphometry



parameters which, for the purpose of this study, included detected area, number of objects (villi), object area, object breadth, object circularity, object height, object length, object perimeter, elongation, and width (Appendix D). These parameters were measured for each villus within the field. Some of the parameters were based on the derived irregularity factors (A_{FE}, P_{FE}; Appendix D).

This procedure was repeated in a slightly modified manner on the same field so as to determine the trophoblastic area per field. The data collected per field was merged with data obtained from other fields of the same specimen. Then, all data obtained from specimens of like genotype were merged into a single large data file which corresponded to one of the three developmental intervals used in the study (7.3B).

7.3 Results

A. STS Analysis in Various Genotypic Populations

The data were tabulated, the means of STS length, STS width, and the ratio of STS length/STS width were computed for both the normal and heteroploid populations. Student t-tests were used to compare the means which are found in Table 7.1. STS length and the ratio of length/width were statistically insignificant for the combined heteroploid population as compared with the normal.

B. Placental Histomorphometry

Data obtained from the normal control population (16 cases) covered a total area of $1.24 \times 10^4\ \mu^2$ and were compared to the sum analyzed area of each of trisomy 15 ($4.5 \times 10^3\ \mu^2$), trisomy 16 (6 cases; $4.8 \times 10^3\ \mu^2$) and trisomy 22 (7 cases; $5.4 \times 10^3\ \mu^2$). The raw data were analyzed by grouping cases of like genotype into one of three categories: 6-7 weeks DA, 8-10 weeks DA, or 11-13 weeks DA. This, in part, accounts for the larger area of normal placenta examined; more normal cases were require to cover the combined range of developmental arrest in the three study populations. Sufficient cases of trisomy 15, 16, and 22 were not available at 6-7, 11-13, and 8-10 weeks, respectively, to permit analysis of development in those periods. Where data were available, the appropriate data of like genotype were lumped together for analysis. The statistical analysis was completed by obtaining the mean values of each of the previously defined parameters so as to obtain a quantitative description of placental development in these populations.

In comparison to normal development, the data mean values for each parameter (Table 7.2) demonstrate:

1) fewer numbers of villi in each heteroploid population
2) a tendency to increased average villous area in heteroploidy; only the 8-10 week interval in trisomy 15 placentae showed villous area to be equal to that in the corresponding normal placentae

Table 7.1: Mean Ratios of STS Length, STS Width, and STS Length/STS Width in N and Heteroploid Development

Genotype	N	WDA	Mean STS Length	Significance	Mean STS Width	Significance	Mean $\frac{\text{STS Length}}{\text{STS Width}}$	Significance
TA/SA	15	7-13	9.0	-	7.9	-	1.2	-
Heteroploid	4	7-13	10.1	>0.05	7.0	>0.05	1.6	>0.05

SA denotes missed abortions which were morphologically normal.

Table 7.2: Summary of Histomorphometric Means of Various Parameters Taken Over Control and Study Populations

DA (weeks): Study Group:	6-7 Normal	6-7 Trisomy 16	6-7 Trisomy 22	8-10 Normal	8-10 Trisomy 15	8-10 Trisomy 16	11-13 Normal	11-13 Trisomy 15	11-13 Trisomy 22
Area in field (μ^2)	8.6×10^1	8.6×10^1	8.6×10^1	8.6×10^1	8.6×10^1	8.6×10^1	8.6×10^1	8.6×10^1	8.6×10^1
Area Occupied by Villi (μ^2)	4.8×10^5	4.5×10^5	4.3×10^5	4.6×10^5	3.6×10^5	3.8×10^5	4.6×10^5	4.0×10^5	4.3×10^5
Number of Villi	2.0×10^3	1.3×10^1	1.6×10^1	1.8×10^1	1.4×10^1	1.3×10^1	1.9×10^1	1.1×10^1	1.3×10^1
Area per Villus (μ^2)	2.4×10^4	3.6×10^4	2.7×10^4	2.6×10^4	2.6×10^4	3.0×10^4	2.4×10^4	3.8×10^4	3.2×10^4
Villous Breadth (μ)	1.2×10^2	1.6×10^2	1.4×10^2	1.3×10^2	1.3×10^2	1.5×10^2	1.2×10^2	1.5×10^2	1.4×10^2
Villous Circularity	7.9×10^{-1}	7.3×10^{-1}	7.7×10^{-1}	8.1×10^{-1}	7.5×10^{-1}	7.7×10^{-1}	7.9×10^{-1}	6.5×10^{-1}	7.2×10^{-1}
Villous Height (μ)	1.6×10^2	2.0×10^2	1.7×10^2	1.6×10^2	1.9×10^2	1.9×10^2	1.5×10^2	2.0×10^2	1.8×10^2
Villous Length (μ)	2.2×10^2	2.5×10^2	2.2×10^2	2.2×10^2	2.4×10^2	2.5×10^2	2.1×10^2	3.1×10^2	2.6×10^2
Villous Perimeter (μ)	6.3×10^2	7.5×10^2	6.2×10^2	6.3×10^2	6.9×10^2	7.2×10^2	6.2×10^2	8.5×10^2	7.6×10^2
Villous Width (μ)	1.7×10^2	2.0×10^2	1.8×10^2	1.8×10^2	1.8×10^2	2.1×10^2	1.7×10^2	2.7×10^2	2.2×10^2
Villous Elongation	2.1	1.85	1.9	2.0	1.9	1.9	2.1	2.2	2.2
Irregularity Factor A_{EE}	0.8635	0.8722	0.8955	0.8635	0.9420	0.9813	0.8243	0.9606	0.8929
Irregularity Factor P_{EE}	1.18	1.17	1.10	1.15	1.19	1.15	1.20	1.18	1.21
Total trophoblastic Area (μ^2)	1.4×10^5	9.0×10^4	1.2×10^5	1.2×10^5	1.0×10^5	1.0×10^5	1.2×10^5	9.0×10^4	1.2×10^5

3) a tendency toward increased villous breadth, length and height; the only exceptions were breadth in trisomy 15 (6-7 weeks DA) and length in trisomy 22 (6-7 weeks DA)
4) no consistent change in elongation (length/breadth)
5) reduced circularity
6) generally increased irregularity factor
7) a marked reduction in trophoblastic area; the exception was trisomy 22 (11-13 weeks)

7.4 Interpretation of Results

The phenotypic variability associated with heteroploid conceptuses affects the STS analysis conducted in this component. Non-molar triploid placentae like the non-molar placentae examined in this component show reduced villus branching and loose packing; these traits may also be associated with trisomies 13 and 18, although these trisomic conceptuses may also have near normal presentation. The data demonstrate a insignificant changes in the STS length/width ratio from insignificant changes in STS length and width. This finding may be explained by phenotypic variability accounting for variable placental thicknesses in heteroploidy. Variations in placental thickness will impede statistical detection of truncal system changes, as will the small sizes of the systems.

A significant decrease in truncus width was expected but not found. This may be an effect of the small heteroploid sample size and the fact that the trisomic systems analyzed may have approximated normal development (Table 5.1). The mean value of STS width for the heteroploid population was reduced, and for this reason, it would be advisable to conduct further similar analyses with larger sample sizes which would permit analysis based on specific genotypes like 3n, trisomy 13 and trisomy 18.

Other forms of heteroploidy were analyzed by the semi-automated interactive morphometric program Genias 25 (Joyce Loebl). The limited use of similar programs in placental quantitation has been applied previously in studies involving various numbers of term placentae, i.e., Bouw *et al.* (1978) - 37 cases; Franco *et al.* (1987) - 200 cases; and Teasdale and Jean-Jacques (1988) - 15 cases. The present, essentially preliminary study on previable placentae included 35 cases.

The results were successful in corroborating gross and microscopic phenotypic changes associated with previable diploid and heteroploid placentation. Specifically, the heteroploid placentae showed looser packing as indicated by:

1) fewer numbers of villi
2) reduced area occupied by villi within the field

Also, hydropic changes in villous stroma and failure of branching into smaller villi were demonstrated by:

1) increased area per villus
2) increased villous breadth, length, height, and width
3) increased villous perimeter

Irregularity in villous shapes as identified by a combination of:

1) reduction in villous circularity
2) increased villous perimeter
3) increased villous irregularity factor A_{EE} except in the early period

The relationship between circularity, area and perimeter is simple. As circularity decreases, the object becomes less regular, hence, less circular and the greater is the object perimeter. The result of increased perimeter and reduced circularity is reduced area. The fact that no difference in villous irregularity was demonstrated over the early interval, may be a result of small numbers and the possibility that the degree of villous irregularity at this period is small and it takes time to become obvious. Finally, a general reduction in trophoblastic area indicative of reduced growth potential was demonstrated which is strongly associated to conceptal phenotype in heteroploidy.

Certain alternatives existed with respect to the histomorphometric analysis that may have improved the range of data obtained from these placental sections. The protocol employed in this component of the thesis was a simple one that paralleled that of other studies analyzing human term placentae. Ten complete fields were analyzed in most cases; sufficient material was not available in three cases, necessitating the analysis of fewer than ten fields. As a result of analyzing complete fields, villi which were hanging over the edges of the fields gave values which were not truly representative of those villi. Consequently, the quantitative data obtained must be interpreted bearing this in mind. The error generated in these analyses was consistent and therefore not significant, making this an acceptable morphometric approach. By analyzing entire fields, insight is gained into general placental development.

An alternative approach to complete field analysis would be to measure given numbers of stem, intermediate, and peripheral villi in order to obtain statistics on each at given developmental ages. This would provide more information on the development of each type of villus, and permit their direct comparison amongst diploid and heteroploid populations which cannot be achieved via complete field analysis. The drawback to this method is that it provides no information as to the density of the field, and consequently, the placenta and its overall development.

Both approaches are useful in providing complementary, non-identical information regarding placental development. It was not practical to utilize the latter approach in the present study due to time constraints. Nonetheless, the unique information potentially available by such analyses would suggest that it is worth investigating. No other study has attempted to analyze placental development through this approach. The semi-automated histomorphometric system is a promising research tool in quantitative placental analysis.

CHAPTER 8

GENERAL DISCUSSION

8.1 Phenotype-Genotype Correlations: Expanding the Spectrum

Phenotype-genotype correlations are complicated by the expression of non-specific traits. This makes the spectrum associated with different genotypes largely unpredictable, although in the absence of cytogenetics one may be able to clinically recognize forms of heteroploidy based on clusters of traits and not absolute phenotype.

The component of this thesis which attempted to expand the phenotypic spectra associated with different forms of heteroploidy in the previable period, was successful in corroborating and expanding these spectra based on a thorough pathological examination entailing careful gross and microscopic observation. In light of reductions in hospital services, and specifically in the availability of karyotypic analyses for spontaneous abortions, it is evident that there will be fewer opportunities for expanding the phenotypic spectrum associated with heteroploidy as expressed in the previable period. Furthermore, studies implicating the observer's lack of familiarity causing inconsistencies in the qualitative evaluation of products of conception (Minguillon *et al.*, 1989), and the non-specificity of phenotypes, especially those associated with placental development, bring into question the value of examining these tissues (Fox, 1993).

With respect to embryonic/fetal development, new contributions to the spectra included, amongst others, the identification of diastasis recti in trisomies 13, 18, and 21, iniencephaly with complete rachischisis and agenesis of the corpus callosum and olfactory bulbs in trisomy 18, and the identification of a formed, pure trisomy 22 embryo with external features which parallel to those of a previously described neonate (Kukolich, 1989). Internal examination of this embryo revealed profound disturbances affecting multiple systems, mostly derived from the mesoderm. The study confirmed reports of embryonic findings in development of non-viable trisomies and embryonic/fetal descriptions in the potentially viable populations (trisomies 13, 18, and 21, 45,X).

The classifications of many of these conceptuses, especially the non-viable trisomies, was problematic because the recovered tissue from these spontaneous abortuses was often a ruptured chorionic sac with evidence of early blighting. As a result, if there is an absence of fetal nucleated red blood cells in chorionic vessels, the conceptus is classified as GD_1 (Poland and Miller, 1973). However, difficulties arise with ruptured chorionic sacs which contain nucleated red blood cells on histologic examination. The presence of nucleated red blood cells automatically classifies the conceptus as GD_2 or better (developmental arrest at 5-6 weeks).

Multiple contributions were made with respect to placental analysis primarily in the form of more complete placental descriptions. Trisomies 6 and 19, and monosomy 21 provided novel contributions to the literature; this was the first documented case of

trisomy 6 which has a placental description, and the third reported case of monosomy 21, but the first to associate monosomy 21 with partial mole.

Previous descriptions of diploid, trisomic, triploid and tetraploid placental development were confirmed. Examination of these populations demonstrated that the lack of phenotypic specificity also extends to the placenta. For example, although extreme placental hypoplasia and other traits outlined by Boué *et al.* (1985) are usually associated with heteroploidy, they may be present in the diploid placenta. Even intrastromal trophoblastic buds caused by deep trophoblastic invagination, and intravillous cytotrophoblastic cells were observed in one of the diploid cases in this study.

Furthermore, this illustrates the fact that not all diploid specimens are normal. For example, the diploid study population used in qualitative assessment involved 59 cases of growth disorganized embryos, 12 cases of structurally abnormal formed embryos and fetuses, and 14 partial and 6 complete hydatidiform moles. It is acceptable to suggest that most, but definitely not all diploid cases would be normal. This fact is worth stressing because it has been taken for granted in previous semi-quantitative studies where the only criterion for inclusion in a control population has been diploidy.

Separating the concept of normal from diploid and identifying the abnormal and the heteroploid can only be achieved by careful examination of all available tissues. The morphological basis of distinction amongst these groups can be subtle or obvious as determined in this study. The fundamental distinction between normal and abnormal is structural anomaly which, in externally structurally normal fetuses, will occur internally in less than 1% of cases. Structural anomaly is occasionally seen in diploid conceptuses, but is far more frequent in heteroploidy. In fact, none of the heteroploid conceptuses analyzed in this study exhibited normal development. Furthermore, a large series of cases in this study were cytogenetically-confirmed cases of diploidy. Cases such as these, which mimic the expression of another genetic population, are termed phenocopies (Nora and Fraser, 1989).

The occurrence of phenocopies, and the continuum of phenotypes associated with genotype create the phenotypic overlap responsible for ambiguity in conceptal identification in the absence of cytogenetics. Consequently, in light of the expression of the phenotypic spectrum associated with cataloguing observations of these cases, it is possible to more accurately suggest the heteroploid origin of a conceptus, but not to do so with complete accuracy; ultimately, karyotyping is still required to achieve this.

For this reason, and for the specific reason of understanding abnormal human development, it is important to record and expand the phenotypic spectrum associated to heteroploidy. This is perhaps even more important now at a time of fiscal restraint when the availability of karyotypic analysis on spontaneous abortuses is reduced.

8.2 The Science of Anthropometry and its Implications to Post-Mortem Analysis of the Previable Conceptus

The quantification of early human development was achieved by anthropometry and histomorphometry. Anthropometry was used in this thesis to identify changes in

growth regulation across the potentially viable forms of heteroploidy (trisomies 21, 13, and 18; 45,X; 3n). Since the quantitation of abnormal development has not been previously attempted over this period of gestation (<20 weeks), it could not be used to corroborate the findings of previous quantitative studies although it could be employed as a comparison with other phenotypic populations.

Using this method, tight linear correlations were demonstrated in normal development. The same was demonstrated for most parameters in the heteroploid populations, which is responsible for the symmetric growth with focal abnormalities typical of these populations. Retention-associated changes were found to interfere with the cranium due to softening of the brain leading to secondary collapse; severely macerated fetuses with cranial distortion, should be employed in anthropometry so as to obtain limited measurements avoiding the cranial region.

In comparison, the trisomy 21 population demonstrated reduced growth of the calvarial volume, reduced nasal index, increased growth rate of nasal width, reduced growth rate of the aural length, increased variability of IPD/BPD and IPD/FW, and decreased facial index. The variances of trisomy 21 developmental parameters were similarly affected; the variances of calvarial volume, cranial index, cervical index, aural length and cervical length were increased. In addition, a reduced growth rate of the complete upper arm, forearm and feet was demonstrated. All of these findings in trisomy 21 correlated with phenotypic observations in this population which included frequent lack of occipitofrontal bossing, wide nose, round ears, normal to increased intercanthal distance, round face and head, and short upper limbs. Furthermore, greater variability was observed to affect certain craniofacial and cervical parameters.

The 45,X population demonstrated an increased variance in the aural width accompanied by decreased growth rates of both the aural length and width; the growth rate of the upper lip was also reduced. The findings pertaining to aural development are consistent with the oblong shape of the ear in 45,X. Furthermore, changes in chest circumference and cervical width were observed which are related to the degree of lymphedema and cystic hygroma in the fetus.

In the other experimental populations, the triploid fetuses demonstrated reduced growth rate of the upper limbs whereas no significant changes were identified for the trisomy 13 and 18 populations.

The anthropometric analysis of these populations was valuable because it provided information regarding subtle developmental changes in these conceptuses. Lack of expertise and loss of interest in this field led to reduced critical, quantitative analysis of spontaneous and therapeutic abortuses such as these, and make further similar analyses unlikely. Nonetheless, these data are useful because they provide a comprehensive survey of human development during the first and second trimesters; the methodology is easily performed in a relatively inexpensive manner by a minimally trained investigator.

The second phase of quantitation of development involved placental parameters which were measured grossly and histomorphometrically. The single truncal system method of gross placental analysis demonstrates that simple systems like placental villus "trees" reveal the extent of truncal branching, and thus, growth potential of the placenta.

Since the gross analysis of the placenta does not provide such close definition of the truncal systems, single truncal system analysis should not be overlooked. As a building block in placental analysis, however, STS analysis has only been conducted once before (Bøe, 1953).

The next level of quantitative placental assessment was that of histomorphometry. Being the first study of its kind in early abortuses, this preliminary study also confirmed placental phenotype but to do so, applied quantitative measures to the histologic presentation. The presentation of large hydropic villi with trophoblastic hypoplasia, and a loosely packed placenta with fewer numbers of villi is typical of the trisomies examined; this was confirmed histomorphometrically.

Generally this method is time-consuming but useful in quantitation. The trade-off between time and quantitation diminishes its value to the routine analysis of the placenta as in diagnostic pathology, but makes it a very reasonable research instrument which can be further enhanced by use of an automated stage in order to avoid potential bias in sampling.

8.3 Quantitation of Placental Patterns

Expansion of phenotypic spectra can only be achieved with an understanding of genotype and the recognition of phenotype. Once again, both are dependent on careful, and deliberate observation, and when combined may permit the verification of qualitative traits by quantitative methods. This was the case with several of the observations of this study.

In the trisomy 21 population, qualitative findings of short limbs, lack of occipitofrontal bossing, round ears and hypertelorism were confirmed by anthropometry. A novel qualitative finding was that neural tube defects do not appear to be associated with trisomy 21, as far as could be ascertained from this study population.

The 45,X population demonstrated the presence of oblong ears and a reduced philtrum, both of which were confirmed by anthropometry; there also appeared to be an association of these malformations with cystic hygroma. Thus, the phenotype of a 45,X conceptus was more severely affected if a cystic hygroma was present.

Other heteroploid populations such as trisomies 14, 15 and 22 showed hypoplastic placentation without genotype-specific findings. The non-viable trisomies exhibited generalized villous hypoplasia, loss of villous structural uniformity and disturbed morphogenesis with mixtures of compact and clubbed or cystic villi. In addition, trisomy 22 showed the presence of deep trophoblastic invaginations (intrastromal trophoblastic buds). Complete placental descriptions of other heteroploid conceptuses were also provided by this study.

Ultimately it is hoped, the recognition of qualitative, morphological traits which are verifiable quantitatively will be improved based on molecular data on chromosomal loci. Studies involving animal models of human aneuploidy, established on sequence homology of gene products (Epstein *et al.*, 1987) will be beneficial in this analysis, and will possibly identify human homeobox regions which are integral to early human organo- and morphogenesis.

8.4 Amplified Developmental Instability vs. Specific Gene Effect

The trisomy 21 population was evaluated, in part, to allow for analysis of variance in metric traits leading to amplified developmental instability. As per Shapiro's (1983) contention, it was expected that traits which exhibit the greatest variability in normal human development would show greater variability in the aneuploid population due to the integrated action of excess gene product from loci of chromosome 21 with those from the remainder of the genome. A reduced ability to respond and tolerate these changes leads to the Down syndrome phenotype with which we have become familiar.

In comparison, Epstein's (1986) reductionist theory implicated individual loci in the formation of the trisomy 21 phenotype since single anomalies can be mapped to individual loci within the genome. The ability to corroborate such a theory is only possible if one can discern the functions of any genes located on the extra chromosome. However, Epstein readily states that he does not believe all traits will be mapped to individual loci, suggesting that some will certainly result from interactions. Nonetheless, the molecular nature of this theory placed it beyond the scope of this thesis; neither were specimens included in this study representative of single locus mutations, nor were they analyzed for variations associated to specific traits within existing loci.

In so far as it was possible to test the hypothesis with respect to variation in metric traits, the data did not demonstrate that parameters showing greater variation in normal development did so in trisomy 21. Thus, the data did not support the hypothesis of amplified developmental instability.

8.5 Consequences of This Project

The relationship between genotype and phenotype is a continuum influenced by interactions within the genome itself, stochastic possibilities of whether alleles, genes and chromosomes will be inherited by the conceptus, and environmental factors. Due to the complex nature of this association, the analysis of human development is increasingly complicated.

Despite the complexity and perceived difficulty, it is necessary to acknowledge that the very basis of all studies in human development is simple morphologic analysis. Only from the establishment of such a qualitative framework of observations, can a greater depth of developmental study be undertaken.

Morphologic analyses enable the researcher to pursue anthropometric studies at the fundamental level of quantitation. This relatively simple methodology permits the observer insight into the physiological nature of morphologic observations.

The combined understanding of morphologic and anthropometric analyses justify the careful, routine analysis of all aborted embryos, fetuses and respective placentae. Furthermore, with advances in molecular biology which will associate individual loci with specific gene products and their functions, these analyses will be of vital scientific importance by providing exclusive data for correlation to results of experimental studies.

CHAPTER 9

CONCLUSION

The purpose of this thesis was to expand the phenotypic spectrum of different types of heteroploidy while also quantitatively analyzing embryo/fetal and placental growth. It was demonstrated that the non-viable aneuploidies, which arrest development in the first trimester, show a very narrow overlapping phenotypic spectrum. In comparison, the potentially viable forms of heteroploidy show a greater spectrum. Additionally, it was shown that anthropometry is a useful and reliable tool in the pathological quantitative analysis of anomalous human development; these findings tend to correspond closely with both the subtle and obvious phenotypic presentations of these conceptuses. Furthermore, the value of quantitatively examining placental truncal systems and histologic sections was also demonstrated using the dissecting microscope and a computerized, semi-automated image analysis system run with Genias 25 software (Joyce Loebl), respectively. This study was preliminary in nature, being the first such study of placentae comprised of several different genotypic populations at this developmental stage. Although this was not a specific objective of the thesis, the value of examining abortuses, whether spontaneous or therapeutic, has been demonstrated; the information concealed in this tissue is of importance to many, from mother and physician, to researcher and science.

BIBLIOGRAPHY

Baldwin VJ. (1982): Morphologic pathology of fetomaternal interaction. Contrib Gynec Obstet. 9: 1-16.

Barden HS. (1980): Fluctuating dental asymmetry: a measure of developmental instability in Down Syndrome. Am J Phys Anthropol. 52: 169-173.

Berger GS, Edelman DA, Kereniji TD. (1975): Fetal crown-rump length and biparietal diameter in the second trimester of pregnancy. Am J Obstet Gynecol. 122: 9-12.

Bird AP. (1984): DNA Methylation - How important is gene control? Nature. 307: 503-504.

Birkbeck JA, Billewicz WZ, Thompson AM. (1975a): Fetal growth from 50-150 days of gestation, in relation to crown-heel length. Ann Hum Biol. 2: 173-178.

Birkbeck JA, Billewicz WZ, Thompson AM. (1975b): Fetal growth from 50-150 days of gestation. Ann Hum Biol. 2: 319-326.

Bøe F. (1967): Studies on the Human Placenta. I. The cell islands in the young placenta. Acta Obstet Gynec Scand. 46: 591-603

Bøe F. (1968): Studies on the human placenta. II. Gross Morphology of the foetal structures in the young placenta. Acta Obstet Gynec Scand. 47: 420-435

Boué A, Boué J. (1973): Evaluation des erreurs chromosomiques au moment de la conception. Biomed. 18: 372-374.

Boué A, Boué J. (1985): Cytogenetics of pregnancy wastage. In: Advances in Human Genetics. Harris H, Hirschhorn, K (eds). Plenum Press. New York and London. 1-57.

Boué J, Daketsé MJ, Deluchat C, Ravisé N, Yvert F, Boué A. (1976a): Identification par les bandes Q et G des anomalies chromosomiques dans les avortements spontanés. Ann Génét. 19: 233-239.

Boué J, Philippe E, Giroud A, Boué A. (1976b): Phenotypic expression of lethal chromosomal anomalies in human abortuses. Teratology. 14: 3-20.

Bouw et al. (1978): Quantitative morphology of the placenta: III. The growth of the placenta and its relationship to birth weight. Europ J Obstet Gynec Reprod Biol. 8: 73-76.

Bracken MB. (1987): Incidence and aetiology of hydatidiform mole: and epidemiological review. Br J Obstet Gynecol. 94: 1123-1135.

Brenner WE, Edelman DA and Hendricks CH. (1976): A standard of fetal growth for the United States of America. Am J Obstet Gynecol. 126: 555-564.

Bruyere HJ, Arya S, Kozel JS, Gilbert EF, Fitzgerald JM, Reynolds JF Jr, Lewin SO, Opitz JM. (1987): The value of examining spontaneously aborted human embryos and placentas. BDOAS. 23: 169-178.

Byrne J, Blanc WA. (1985): Malformations and chromosome anomalies in spontaneously aborted fetuses with single umbilical artery. Am J Obstet Gynecol. 151: 340-342.

Byrne J, Warburton D. (1979): Some pathologic findings in spontaneous abortions. BDOAS. XV: 137-147.

Byrne J, Warburton D, Kline J, Blanc W, Stein F. (1985): Morphology of the early fetal deaths and their chromosomal characteristics. Teratology. 32: 297-315.

Canki N, Warburton D, Byrne J. (1988): Morphological characteristics of monosomy X in spontaneous abortions. Ann Génét. 31: 4-13.

Carr DH (1967): Chromosomal errors and development. Am J Obstet Gynecol. 104: 327-347.

Carr DH (1969): Cytogenetics and the pathology of hydatidiform degeneration. Obstet Gynecol. 33: 333-342.

Carr DH (1971a): Chromosome studies in selected spontaneous abortions. 3. Early pregnancy loss. Obstet Gynecol. 37: 570-574.

Carr DH (1971b): Chromosome studies in selected spontaneous abortions: Polyploidy in man. J Med Genet. 8: 164-174.

Carr DH, Gideon M. (1977): Population cytogenetics of human abortuses. In: Population Cytogenetics. Hook EB, Porter IH, Eds. Academic Press, New York. pp 1-9.

Cassperson T, Zech L, Johansson C. (1970): Differential binding of alkylating fluorochromesin human chromosomes. Exp Cell Res. 60: 315-318.

Castellucci M, Scheper M, Scheffer I, Celona A, Kauffmann P. (1990): The development of the human placental villous tree. Anat Embryol. 181: 117-128.

Chen ATL, Chan YK, Falek A. (1972): The effects of chromosome abnormality on birth weight in man. Hum Hered. 22: 209-224.

Chervenack FA, Isaacson G, Blakemore KJ, Breg WR, Hobbins JC, Berkowitz RL, Tortora M, Mayden K, Mahoney M. (1983): Fetal cystic hygroma: Cause and natural history. N Engl J Med. 309: 822-825.

Clatworthy HW, Anderson RG. (1944): Development and growth of the human embryo and fetus. Arch Dis Child. 67: 167-175.

Colacino SC, Pettersen JC. (1978): Analysis of gross anatomical variations found in four cases of trisomy 13. Am J Med Genet. 2: 31-50.

Coppoletta, Wolbach (1933): Body length and organ weights of infants and children. Am J Path. 9: 55-70.

Creasy R, Crolla JA. (1974): Prenatal mortality in trisomy 21 (Downs syndrome). The Lancet. I: 473-474.

Creasy MR, Crolla JA, Alberman ED. (1976): A cytogenetic study of human SA using banding technology. Hum Genet. 31: 177-196.

Curry CJ, Honoré LH. (1990): A protocol for the investigation of pregnancy loss. Clin Perinatol. 17: 723-741.

Deter RL, Rossavik IK, Harrist RB, Hadlock FP. (1986): Mathematical modelling of fetal growth: Development of individual growth. Obstet Gynecol. 68: 156-161.

Doerfler W. (1983): DNA methylation and gene activity. Ann Rev Bioch. 52: 93-124.

Eiben B, Bartels I, Bähr-Porsch, Borgmann S, Gatz G, Gellert G, Goebel R, Hammans W, Hentemann M, Osmers R, Rauskolb R, Hansmann I. (1990): Cytogenetic analysis of 750 spontaneous abortions with the direct preparation method of chorionic villi and its implication for studying genetic causes of of pregnancy wastage. Hum Genet. 47: 656-663.

Eiben B, Borgmann S, Schiibbe I, Hansmann I. (1987): A cytogenetic study directly from chorionic villi of 140 spontaneous abortions. Hum Genet. 77: 137-141.

Epstein CJ. (1986): In: The Consequences of Chromosome Imbalance. Principles, Mechanisms, and Models. New York: Cambridge University Press.

Epstein CJ. (1988): Specificity versus nonspecificity in the pathogenesis of aneuploid phenotypes. Invited Editorial Comment. Am J Med Genet. 29: 161-165.

Epstein CJ, Avraham KB, Lovett M, Smith S, Elroy-Stein O, Rotman G, Bry C, Groner Y. (1987): Transgenic mice with increased Cu/Zn-superoxide dismutase activity: Animal model of dosage effects in Down Syndrome. Proc Natl Acad Sci USA. 84: 161-165.

Fantel AG, Shepard TH (1985): Morphological analysis of spontaneous abortuses. pp. 8-28. In: Spontaneous and Recurrent Abortions. Bennett MJ, Edmonds DK (eds) Blackwell Scientific Publications, Oxford.

Fantel AG, Shepard JH, Vadheim-Roth C, Stephens TD, Coleman C. (1980): Embryonic and fetal phenotypes: prevalence and other associated factors in a large study of spontaneous abortion. Pediatr Path. 69: 71-87.

Fisher RA, Lawler SD. (1984): Heterozygous complete hydatidiform moles: Do they have a worse prognosis than homozygous complete moles? Lancet. ii: 51.

Fitzsimmons J, Chinn A, Shepard TH. (1988): Normal length of the human fetal gastrointestinal tract. Ped Path. 8: 633-641.

Foussereau S, Phillipe E. (1972): Les produits d'avortements tardif: Fréquence des aberrations chromosomiques. J Gyn Obst Biol Repr. I: 651-656.

Fox H. (1985): Placental pathology: A contemporary approach. Obstet Gynecol Annual. 14: 427-440.

Fox H. (1986): Pathology of the placenta. Clin Obstet Gynecol. 13: 501-519.

Fox H. (1993): Histological classification of tissue from spontaneous abortions: A valueless exercise? Histopathology. 22: 599-600.

Franco V, Aragora F, Stella M, Florena AM, Raimondi L. (1987): Computerized interactive morphometry as a potentially useful tool for the study of placenta. Acta Europaea Fertil. 18: 259-261.

Geisler M, Kleinebrecht J. (1978): Cytogenetic and histopathologic analyses. Humangenetik. 45: 239-251.

Gilbert Ef, Arya S, Laxova R, Opitz JM. (1987): Pathogenesis of chromosomal abnormalities in the fetus - pathologic markers. BDOAS. 23: 293-306.

Ginsberg J, Bofinger ATK, Rousch JR. (1977): Pathologic features of the eye in Down's syndrome with relationship to other chromosomal anomalies. Am J Opthamol. 83: 874-880.

Giroud A, Deluchat CH, Boué J. (1974): Chromosomal aberrations and abnormalities, especially of the prosencephalon. C R Seances Soc Biol Fil. 168: 210-213.

Golbus MS, Berry LL. (1976): Human fetal development between 90 - 170 days postmenses. Teratology. 15: 103-108.

Gruenwald P, Minh HN. (1960): Evaluation of body and organ weights in prenatal pathology. Am J Pathol. 34: 247-253.

Hadlock FP, Harrist RB, Sharman RS, Deter RL, Park SK. (1985): Estimation of fetal weight with the use of head, body and femur measurements - a prospective study. Am J Obstet Gynec. 151: 333-337.

Hassold TJ, Chen N, Funhouser J, Jooss T, Manuel B, Matsuura J, Matsuyama A, Wilson C, Yamane JA, Jacobs PA. (1980): A cytogenetic study of 1000 abortions. Ann Hum Genet. 44: 151-164.

Hassold TJ, Jacobs PA. (1984): Trisomy in man. Ann Rev Genet. 18: 69-97.

Hassold TJ, Pettay D, Freeman SB, Grantham M, Takaesu N. (1991): Molecular studies of non-disjunction in trisomy 16. J Med Genet. 28: 159-162.

Hern WM. (1984): Correlation of fetal age and measurements between 10-26 weeks of gestation. Obstet Gynecol. 63: 26-32.

Hill LM, Brackle R, Wolfgram KR. (1983): An ultrasonic view of the developing fetus. Obstet Gynecol Surv. 38: 375-398.

Honoré LH, Dill FJ, Poland BJ. (1976): Placental morphology in spontaneous human abortion with normal and abnormal karyotypes. Teratology. 14: 151-166.

Honoré LH, Robertson JA. (1985): A basic protocol for the laboratory investigation of human spontaneous abortion. Infertility. 8: 227-234.

Iffy L, Shepard TH, Jakobovits A, Lemire RJ, Kerner P. (1967): The rate of growth in young human embryos of Streeter's horizons XIII to XXIII. Acta Anat. 66: 178-186.

Iffy L, Jakobovits A, Westlake W, Wingate M, Caterini H, Kanofsky P, Menduke H. (1975): Early intrauterine development: I. The rate of growth of caucasian embryos and fetuses between the 6th and 20th weeks of gestation. Pediatrics. 56: 173-186.

Iffy L, Lavenhar M, Kaminetzky HA, Jongbloet PH, Wingate MB, Jakobovits A. (1978): The effect of maternal age, parity, fetal sex and season upon early intrauterine development. Int J Gynaecol Obstet. 16: 210-224.

Illsey NP, Fox H, Van der Veen F, Chawner L, Penfold P. (1985): Human placental ultrastructure after in vitro dual perfusion. Placenta. 6: 23-32.

Isada NG, Bolan JC, Larsen JW, Kent SG. (1990): Trisomy 22 with holoprosencephaly: A clinicopathological study. Teratology. 42: 333-336.

Jackson CM. (1909): On the perinatal growth of the human embryo and the relative growth of the various organs and parts. Am J Anat. 9: 119-165.

Jacobs PA. (1979): Recurrence risks for chromosome abnormalities. BDOAS. 15: 71-80.

Jakobovits A, Westlake W, Iffy L, Wingate M, Caterini H, Chatterton R, Lavenhar M. (1976): Pediatrics. 58: 833-841.

Kajii T. (1980): Androgenetic origin of hydatidiform moles: its bearing on carcinogenesis. Gann Monoogr Cancer Res. 25: 189-194.

Kajii T, Ferrier A, Niikawa N, Takahara H, Ohama K, Avirachan S. (1980): Anatomic and chromosomal anomalies in 639 spontaneous abortuses. Humangenetik. 55: 87-98.

Kajii T, Ohama K. (1977): Androgenetic origin of hydatidiform mole. Nature. 268: 633-634.

Kajii T, Ohama K, Niikawa N, Ferrier A, Avirachan S. (1973): Banding analysis of abnormal karyotypes in spontaneous abortion. Hum Genet. 25: 539-547.

Kajii T, Ohama K, Mikamo K. (1978): Anatomic and chromosomal anomalies in 944 induced abortuses. Hum Genet. 43: 247-258.

Kalousek DK. (1987): Anatomical and chromosomal anomalies in specimens of early spontaneous abortion: Seven year experience. BDOAS. 23: 153-168.

Kalousek DK, Barrett IJ, McGillivrary BC. (1989): Placental mosaicism and intrauterine survival of trisomies 13 and 18. Am J Hum Genet. 44: 338-343.

Kelly TE, Ferguson JE, Golden W. (1992): Survival of fetuses with 45,X: An instructive case and an hypothesis. Am J Med Genet. 42: 825-826.

Kleinebrecht J, Geisler M. (1975a): Chromosome aberrations as a cause of spontaneous abortion. Zeitschrift fur Allgemeinmedizin. 51: 974-977.

Kleinebrecht J, Geisler M. (1975b): Histologic analysis of spontaneous abortions with trisomy 2: First description of an embryo. Humangenetik. 29: 15-22.

Kucera J, Dolezalova V. (1972): Prenatal development of malformed fetuses at 28-42 weeks of gestational age. Biol Neonate. 20: 253-261.

Kuhlman RS, Werner AL, Abramowicz J, Warsol SL, Arrington J, Levy DL. (1990): Placental histology in fetuses between 18 and 23 weeks' gestation with abnormal karyotype. Am J Obstet Gynecol. 163: 1264-1270.

Kukolich MK, Kulharya A, Jalal SM, Drummond-Borg M. (1989): Trisomy 22: No longer an enigma. Am J Med Genet. 34: 541-544.

Kuliev AM. (1971): Cytogenetic investigation of spontaneous abortion. Humangenetik. 12: 275-283.

Kurjak A, Kirkinen P. (1982): Ultrasonic growth pattern of fetuses with chromosomal abnormalities. Acta Obstet Gyne Scand. 61: 223-225.

Lauritsen JG, Jonasson J, Therkelsen AJ, Lass F, Lindsten J, Petersen JB. (1972): Studies on spontaneous abortions. Fluorescence analysis of abnormal karyotypes. Hereditas. 71: 160-163.

Lauritsen JG. (1976): Aetiology of spontaneous abortion. A cytogenetic and epidemiological study of 288 abortuses and their parents. Acta Obstet Gynecol Scand Supp. 52: 87-98.

Lawler SD, Fisher RA. (1987): Genetic studies in hydatidiform mole with clinical correlations. Placenta. 8: 77-88.

Lee LG, Jackson JF. (1972): Diagnosis of Downs Syndrome: Clinic versus Laboratory. Clin Ped. 11: 353-356.

Lerner IM. (1954): Book: Genetic Homeostasis Dover Publicus, Inc., NY. Part III Interpretation. pp 63-134.

Levine C. (1989): Primary disorders of the lymphatic vessels - a unified concept. J Ped Surg. 24: 233-240.

Lewis AJ. (1964): The pathology of 18 trisomy. J of Ped. 65: 92- 101.

Lin CC, DeBraeckeleer M, Jamro H. (1985): Cytogenetic studies in spontaneous abortion: the Calgary experience. Can J Genet Cytol. 27: 565-570.

Mall FP. (1918): On the age of human embryos. Amer J Anat. 23: 397-422.

McBride ML, Baillie J, Poland BJ (1984): Growth parameters in normal fetuses. Teratology. 29: 185-191.

Meaney FJ, Farrer LA. (1986): Clinical Anthropometry and Medical Genetics: A compilation of body measurements in genetic and congenital disorders. Am J Med Genet. 25: 343-359.

Mikamo K (1970): Anatomic and chromosomal anomalies in spontaneous abortion. Am J Obstet Gynecol. 106: 243-254.

Minguillon C, Eiben B, Bähr-Porsch S, Vogel M, Hansmann I. (1989): The predictive value of chorionic villus histology for identifying chromosomally normal and abnormal spontaneous abortions. Human Genet. 82: 373-376.

Moore GW, Hutchins GM, O'Rahilly R. (1981): The estimated age of staged human embryos and early fetuses. Am J Obstet Gynecol. 139: 500-506.

Mukerje B. (1980): Measurements on the human fetal uterus and ovary. Acta Obstet Gyne Scand. 59: 549-550.

Nishimura H, Takano K, Tanimura T, Yasuda M. (1968): Normal and Abnormal Development of Human Embryo: First report of the analysis of 1,213 intact embryos. Teratology. 1: 281-290.

Nora JJ, Fraser FC (1989): Medical genetics: Principles and practice. 3Ed. Lea & Febiger. Philadelphia.

Novak R, Agamanolis D, Dasu S, Igel H, Platt M, Robbinson H, Shehata B. (1988): Histologic analysis of placental tissue in spontaneous abortion. Pediatr Pathol. 8: 477-482.

O'Rahilly R. (1967): Normal development of the human embryo. In: Normal and Abnormal Embryological Development. Frantz CH (ed). Nat Res Coun. Nat Acad Sci, Washington, D. C., pp 3-15.

Ohama K, Kajii T, Okamoto E, Fukuda Y, Imaizumi K, Tsukahara M, Kobayashi K, Hagiwara K. (1981): Dispermic origin of XY hydatidiform moles. Nature. 292: 551-552.

Ohama K, Katsunori U, Okamoto E, Takenaka M, Fujiwara A. (1986): Cytogenetic and clinicopathologic studies of partial moles. Obstet Gynecol. 68: 259-262.

Pedersen JF, Mantoni M. (1983): The prognostic significance of fetal size in early diabetic (and normal?) pregnancy and in threatened abortion. Ultrasound Med Biol. Suppl 2: 573-576.

Penrose, Delhanty (1961): Triploid cell cultures from a macerated fetus. The Lancet. I: 1261

Perry TB, Bennie RJ, Cassar N, Hamilton EF, Stocker J, Toftager-Larsen K, Lippman A. (1984): Fetal cephalometry by ultrasound as a screening procedure for the prenatal detection of Down's Syndrome. Br J Obstet Gynec. 91: 138-143.

Petersen MB, Hansen M, Djernes BW. (1987): Full trisomy 22 in a newborn infant. Ann Genet. 30: 101-104.

Petrikovsky BM, Shmoys SM, Baker DA, Monheit AG. (1991): Pleural effusion in aneuploidy. Am J Perinat. 8: 214-216.

Philippe E, Boué JG. (1969): Le placenta des aberrations chromosomiques létales. Ann Anat Path (Paris). 14: 249-269.

Pi SY, Fineman RM, Wing SD, Grunnet M, Chan G. (1980): Holoprosencephaly in a Down syndrome child. Amer J Med Genet. 5: 201-206.

Plachot J, de Grouchy J, Junca AM, Mandelbaum J, Turleau C, Couillin P, Cohen J, Salat-Baroux J. (1987): From oocytes to embryo: A model, deduced from in vitro fertilization for natural selection against chromosome abnormalities. Ann de Genet. 30: 22-32.

Poland BJ, Miller R. (1973): Effect of karyotype on zygotic development. In: Les Accidents Chromosomiques de la reproduction. Boué A, Thibault C (eds). INSERM, Paris, pp. 111-118.

Poland BJ, Miller R, Harris M, Livingston J. (1981): Spontaneous abortion: A study of 1961 women and their conceptuses. Acts Obstetricia et Gynecologica Scandinavica. Supp 102: 4-32.

Quinlan RW, Brumfield C, Martin M, Cruz AC. (1982): Ultrasonic measurement of femur length as a predictor of fetal gestational age. J Reprod Med. 27: 392-394.

Rehder H, Coerdt W, Eggers R, Klink F, Schwinger E. (1989): Is there a correlation between morphological and cytogenetic findings in placental tissue from early missed abortions? Hum Genet. 82: 337-385.

Roberts DK. (1993): Medical legal aspects of placental examination. Editorial. Obstet Gynecol Surv. 48: 777-778.

Rockelein G, Ulmer R, Schroder J. (1989): Correlation of karyotype and placental morphology in early abortion. Pathologe. 10: 306-314.

Rushton DI. (1984): The classification and mechanism of spontaneous abortion. Perspec Pediatr Pathol. 8: 269-287.

Ruzicska P, Czeilzel A. (1970): Cytogenetic studies on mid-trimester abortuses. Humangenetik. 10: 273-297.

Saji F, Tokugawa Y, Kimura T, Kamiura S, Nobunaga T, Azuma C, Tanizawa O. (1989): A new approach using DNA fingerprinting for the determination of androgenesis as a cause of hydatidiform mole. Placenta. 10: 399-405.

Scammon RE, Calkins LA (1929). The development and growth of the external dimensions of the human body in the fetal period. pp. 31-293. University of Minnesota Press, Minneapolis.

Schultz DM et al. (1962): Weights of organs of fetuses and infants. Arch Pathol. 74: 244-250.

Seeds JW, Cefalo RC. (1982): Relationship of fetal limb lengths to both biparietal diameter and gestational age. Obstet Gynecol. 60: 680-685.

Seeds JW, Cefalo RC, Bowes WA. (1984): Femur length in the estimation of fetal weight less than 1500 grams. Am J Obstet Gynecol. 149: 233-235.

Shami SA, Qadeer T, Schmitt LH, Bittles AH. (1991): Consanguinity, gestational period and anthropometric measurements at birth in Pakistan. Ann Hum Biol. 18: 523-527.

Shapiro BL, Gorlin RW, Redman RS, Bruhl HH. (1967): The palate and Down's syndrome. N Eng J Med. 276: 1460-1463.

Shapiro BL. (1970): Prenatal dental anomalies in mongolism: Comments on the basis and implications of variability. Ann NY Acad Sci. 171: 562-577.

Shapiro BL(1975): Amplified developmental instability in Down's Syndrome. Ann Hum Genet (Lond). 38: 429-437.

Shapiro BL(1983): Down Syndrome - a disruption of homeostasis. Am J Med Genet. 14: 241-269.

Sherwood RJ, Robinson HB, May RL, Meindl RS. (1992): Standardized residuals as a means for detection of growth alteration in the pathologic human fetus. Teratology. 46: 419-427.

Siebert JR (1986): Prenatal growth of the median face. Am J Med Genet. 25: 369-379.

Staflova J. (1971): Relation of the size of the eye to age and length in human ontogenesis. Am J Obstet Gynec. 110: 126-128.

Stephens TD, Shepherd TH. (1980): The Down Syndrome in the fetus. Teratology. 22: 37-41.

Streeter GL. (1920): Weight, sitting height, head size, foot length and menstrual age of the human embryo. Contrib Embryol Carnegie Inst. 11: 143-170.

Surti U, Szulman AE, Wagner K. (1986): Tetraploid partial hydatidiform moles: two cases with a triple paternal contribution and a 92,XXXY karyotype. Hum Genet. 72: 15-21.

Szulman AE, Surti U. (1978a): The syndromes of hydatidiform mole. II.Morphologic evolution of the complete and partial mole. Am J Obstet Gynecol. 132: 20-27.

Szulman AE, Surti U. (1978b): The syndromes of hydatidiform mole. I.Cytogenetic and morphologic correlations. Am J Obstet Gynecol. 131: 665-671.

Tanimura T, Nelson T, Hollingsworth RR, Shepard TH. (1971): Weight standards for organs and early human fetuses. Anat Rec. 171: 227-236.

Tenti P, Rehder H. (1985): Pathology of chromosome abnormality in first trimester embryos. pp. 300-313. In: First Trimester Fetal Diagnosis. Fraccaro M, Simoni G, Brambati B (eds). Springer-Verlag, Berlin.

Tippet P, Kaplan JC. (1985): Report of the committee on the genetic constitution of chromosomes 20, 21, 22. Eighth annual workshop on human gene mapping. Cytogenet Cell Genet. 40: 268-295.

Tsuji K, Yagi S, Nakano R. (1981): Increased risk of malignant transformation of hydatidiform moles in older gravidas: a cytogenetic study. Obstet Gynecol. 58: 351-355.

Vassilakos P, Riotton G, Kajii T. (1977): Hydatidiform mole two entitities. A morphologic and cytogenetic study with some clinical considerations. Am J Obstet Gynecol. 127: 167-170.

Ville Y, Borghi E, Pons JC, Leclorc'h M. (1992): Fetal karyotype from cystic hygroma fluid. Prenat Diagn. 12: 139-43.

Waddington CH. (1942): Canalization of development and the inheritance of acquired characteristics. Nature. 150: 563-565.

Warburton D. (1987): Chromosomal causes of fetal death. Clin Obstet Gynecol. 30: 268-277.

Warburton D, Stein Z, Kline J, Susser M. (1980): Chromosome abnormalities in spontaneous abortion: data from the New York city study. In: Human Embryonic and Fetal Death. Porter IH, Hooke EB (eds). Academic Press, New York, 269-287.

Warburton D, Susser M, Stein Z, Kline J. (1979): Genetic and epidemiologic investigation of spontaneous abortion. BDOAS. XV: 127-136.

Yamashita K, Ishikawa M, Shimizu T, Kuroda M. (1981): HLA antigens in husband-wife pairs with trophoblastic disease. Gynecol Oncol. 12: 68-74.

APPENDIX A: DATA COLLECTION FORMS

THE FOLLOWING ARE THE DATA COLLECTION FORMS USED WITH FRESH OR MACERATED ARCHIVAL AND PROSPECTIVE SPECIMENS.

DATA COLLECTION:
EMBRYO

Specimen _____

Present	**Classification**	Focally
Complete Incomplete	GD 2 GD 3 GD 4	Abnormal Normal

Yolk Sac	**Placenta UC**	**Amniotic Bands**
Absent Present	Absent Absent Present Present	Absent Present

Dimensions:

CR CH	CFH	Fac H CH	DA	DS	OFD	BPD	Head (C)
Eye Loc	Orbit	ICD	Abnorm. A/P		Mouth TD	U Lip A/N W	L Lip A/N W
Palate A/N	Chin A/N	Ear Loc	Pol.	Ear	Abnorm. A/P		UA/LB A/P
Forearm	Hand	Leg/LB A/P	Thigh	Foot	Trunk	Neck (L)	Neck (C)
S. M. A/N	Somites A/P						

External Anomalies: Absent
Present

Craniofacial
Trunk
Upper Limbs
Lower Limbs
Anogenital Zone
Other

DATA COLLECTION:
FETUS

Specimen: _____

Complete Incomplete	Normal Abnormal	Fresh Macerated
Placenta Absent Present	**Yolk sac** Absent Present	**UC** Absent Present

Dimensions:

CRL	CHL	BW	PI	BMI	EDA

Segmental Measurements:

CFH	FH	CH	BPD	OFD	HC
Fontanelle	Neck (L)	Neck (C)	N Fold + / -	Edema 0 1 2 3	CH Absent Present
CH Bilocular Multiloc.	Mouth TD	U Lip A/N W	L Lip A/N W	Palate A/N	Chin A/N
Ears Loc. Ears	LS/N	Abnorm. A/P			
Eyes Loc Orbit Size	ICD EOD	Abnorm. A/P			
Upper Limb U A FA Hand	Fingers Thumb	Abnorm. P'dactyly Syndactyly C'dactyly	Other		
Lower Limb T Leg Foot	Toes	Abnorm. P'dactyly Syndactyly C'dactyly	Other		
Axial Skel Sk. Mus A/N	Spinal Col A/N	Scapulae A/N	Clavicles A/N	Abnorm. A/P	

External Abnormalities: Absent
Present

Craniofac.				
Trunk (L)	Chest (C)	Abdominal (C)	Pelvic (C)	
V Abnorm. Gastro. Omphal.	Other			
D Abnorm. NTD	Other			
Ext. Genit Sex C/P AGD.	Abnorm.			

Anus Perforate Imperf.	Anal Loc A/N Ant/Post	
Umbilicus (C)	Umb. Loc. ˆ P. Sym. ˜ Xiphi.	Abnorm.

DATA COLLECTION
INTERNAL ORGANS

Specimen _____

Placenta Absent Present	**Yolk sac** Absent Present	**UC** Absent Present

A. **CVS**

Heart A/N	L/W	Abnorm.
Vessels A/N	Abnorm.	
Pericardium A/N		

B. Respiratory System

Lungs A/N	Diameter	Weight	Abnorm.
Larynx/Tr/Br A/N	Abnorm.		
Pleura A/N	Abnorm.		

C. Diaphragm

A/N

D. GIS

Eso. A/N	L/Diam	Abnorm.	
Stomach A/N	L/Diam	Abnorm.	
S. bowel A/N	L/Diam	Abnorm.	
L. bowel A/N	L/Diam	Abnorm.	
Liver A/N	3 Diam	Weight	Abnorm.
Biliary System A/N	Abnorm.		
Pancreas A/N	Abnorm.		
Rotation A/N	Abnorm.		

E. Urinary System

Kidneys A/N	L/C	
Ureters A/N	L/C	

Bladder A/N	L/C	
Urethra A/N	L/C	

Specimen _____

F. Genital System

MALE	Testes A/N	L/C	Abnorm.
	Ducts A/N	Abnorm.	
	Prostate A/N	Abnorm.	
FEMALE	Ovaries A/N	L/C	Abnorm.
	Tubes A/N	Abnorm.	
	Uterus A/N	Abnorm.	
	Vagina A/N	Abnorm.	

G. Endocrine System

Thyroid A/N	Diam	Abnorm.
Adrenals A/N	Diam	Abnorm.

H. Lymphoreticular System

Thymus A/N	Size	Abnorm
Spleen A/N	Size	Abnorm

I. Hemopoietic System

NRC A/P	NRC (%) DA
Erythroblastosis	

J. CNS

Fresh/Macerated	Detailed Study Y/N	Dimensions	Weight
Cerebrum A/N	Abnormalities		
Midbrain A/N	Abnormalities		
Hindbrain A/N	Abnormalities		
Ventricles A/N	Abnormalities		

Spinal Cord A/N	Abnormalities

APPENDIX B

INDICES AND SECOND AND THIRD DIMENSION ANTHROPOMETRIC PARAMETERS

Indices used in anthropometric study are as follows:

Conventional

Teloric Index (Orbit/ICD) - direct
Cranial Index (BPD/OFP)

Novel Indices Used in Study

CRL/CHL
Aural Index (Ear Length/Ear Width)
Nasal Index (Nose Length/Nose Width)
Facial Index (Facial Height/Facial Width)
Cervical Index (Neck length/Neck Width)
Interlimb Index (TULL/TLLL)
Tapering Index (CC/AC)
IPD*/BPD and IPD/FW - indirect teloric indices

*IPD = ICD + one orbit width

Human growth, whether embryonic or adult, occurs in three spatial planes. To account for this fact, it was necessary to analyze the relationships of linear parameters with each other. This was achieved by the use of certain conventional parameters like BMI and PI and the development of others like cranial surface area and volume. A list of these parameters follows:

Surface Areas: $\text{Calvarial} = \left(\frac{OFD}{2} \times \frac{BPD}{2}\right) + \pi\left[\frac{(BPD/2) \times CH)}{E}\right] \times \sin^{-1}E$

$$\text{where } E = \sqrt{\left[\frac{(OFD/2)^2 - (BPD/2)^2}{OFD/2}\right]}$$

$$\text{Midfacial Area} = IPD \times FH$$

$$\text{Trunk} = 4/3\pi r^2 h$$

Volumes: $\text{Calvarial} = 4/3\pi\,[(OFD/2) \times (BPD/2) \times CH]$

$$\text{Trunk} = 4/3\pi r^3 h$$

Conventional: BMI, PI

APPENDIX C

CORRELATION COEFFICIENTS OF BMI AND PI

The correlation coefficients involving either parameter BMI or PI were unique since PI consistently gave a negative result whereas BMI, a closely related parameter, gave a positive result.

$$BMI = \frac{\text{weight (kg)}}{\text{height}^2(m^2)} \qquad PI = \frac{\text{weight (kg)}}{\text{height}^3(m^3)}$$

Despite the odd appearance of PI in Table 6.3, the result is expected to show this negative relationship given:

some variable x correlated with $^1/_x \Rightarrow$ negative correlation

On the same basis,

if measurement A is correlated with measurement B as follows:

$$A \text{ correlated with } {}^1/_{B^3} = A\left[\frac{1}{B^2} \times \frac{1}{B}\right] = A\left[\frac{1}{B} \times \frac{1}{B} \times \frac{1}{B}\right]$$

$\left[\frac{1}{B} \times \frac{1}{B}\right.$ is positive such that the remaining correlation is:

$$A\,[{}^1/_B]^a \times [{}^1/_x]$$

therefore, some parameter A [PI ($= \frac{\text{weight}}{\text{height}^3}$)] should yield a negative correlation coefficient.

APPENDIX D

HISTOMORPHOMETRY PARAMETERS

The parameters used in the histomorphometric analysis included the following:

1) Detected Area: total field area based on the number of white pixels on the boundaries of all objects in the field

2) Number of Objects: the number of objects detected in the field

3) Object Area (A_O): the area of each detected object, taking account of any white pixels

4) Object Breadth (B): the Feret (greatest) diameter perpendicular to the length of the object being measured

5) Object Circularity: based on other measurements; equal to 4π Area/Perimeter^2 for that field

6) Object Elongation: computed from object length/object breadth

7) Object Height: the Feret (greatest) diameter vertically

8) Object Length: the greatest object diameter

9) Object Perimeter (P_O): based on the sum of the distances between midpoints of the vectors forming the boundary (which lie in eight 45^o directions)

10) Object Width: the Feret (greatest) diameter horizontally

11) Irregularity Factor $A = \pi\ (L/2 \times B/2)/\ A_O$

12) Irregularity Factor $P = \pi\ (L/2 + B/2)/\ P_O$

APPENDIX E

IRREGULARITY FACTORS - FORMAL PROOF

For any given perimeter, a circle with that perimeter has a maximal area:

Area (circle) = pr^2
Area (ellipse) = pab, where a and b are two radii

For a given parameter, r^2>ab because:

$P= 2pr = p(a+b)$

Therefore, a+b=2r. If a=(r+x), b=(r-x), then:

$ab= (r+x)(r-x) = r^2-x^2$

Therefore, r^2> ab. Furthermore, as the difference between r and (a+b) increases:

$r^2>>ab$

Therefore, as the difference between the axes of an ellipse increases, area decreases.

If we assume villus=ellipse, it has a smooth surface and thus, irregularity = 0.

An ellipse of given diameters maximizes surface area. Thus, as surface irregulariy increases, $A_o<A_{EE}$.

[irregularity factor (villus) = A_{EE}/A_o; irregularity increases as values exceed 1]

A_{EE} = surface area of equivalent ellipse with axes (L, B) of villus

The same calculation may be conducted with perimeter

P_{EE} = perimeter of equivalent eclipse with axes (L,B) of villus

In this situation, this irregularity factor (P_{EE}/P_o) shows greater irregularity as $P_o>P_{EE}$, that is as P_{EE}/P_o approximates zero.